Exhibit 11

Designation: D5755 – 09 (Reapproved 2014)ε1

Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Surface Loading[1]

This standard is issued under the fixed designation D5755; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

ε[1] NOTE—Warning notes were editorially updated throughout in April 2014.

1. Scope

1.1 This test method covers a procedure to (*a*) identify asbestos in dust and (*b*) provide an estimate of the surface loading of asbestos in the sampled dust reported as the number of asbestos structures per unit area of sampled surface.

1.1.1 If an estimate of the asbestos mass is to be determined, the user is referred to Test Method D5756.

1.2 This test method describes the equipment and procedures necessary for sampling, by a microvacuum technique, non-airborne dust for levels of asbestos structures. The non-airborne sample is collected inside a standard filter membrane cassette from the sampling of a surface area for dust which may contain asbestos.

1.2.1 This procedure uses a microvacuuming sampling technique. The collection efficiency of this technique is unknown and will vary among substrates. Properties influencing collection efficiency include surface texture, adhesiveness, electrostatic properties and other factors.

1.3 Asbestos identified by transmission electron microscopy (TEM) is based on morphology, selected area electron diffraction (SAED), and energy dispersive X-ray analysis (EDXA). Some information about structure size is also determined.

1.4 This test method is generally applicable for an estimate of the surface loading of asbestos structures starting from approximately 1000 asbestos structures per square centimetre.

1.4.1 The procedure outlined in this test method employs an indirect sample preparation technique. It is intended to disperse aggregated asbestos into fundamental fibrils, fiber bundles, clusters, or matrices that can be more accurately quantified by transmission electron microscopy. However, as with all indirect sample preparation techniques, the asbestos observed for quantification may not represent the physical form of the asbestos as sampled. More specifically, the procedure described neither creates nor destroys asbestos, but it may alter the physical form of the mineral fibers.

1.5 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

D3195 Practice for Rotameter Calibration

D3670 Guide for Determination of Precision and Bias of Methods of Committee D22

D5756 Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Mass Surface Loading

D6620 Practice for Asbestos Detection Limit Based on Counts

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

[1] This test method is under the jurisdiction of ASTM Committee D22 on Air Quality and is the direct responsibility of Subcommittee D22.07 on Sampling and Analysis of Asbestos.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

3. Terminology

3.1 *Definitions:*

3.1.1 *asbestiform*—a special type of fibrous habit in which the fibers are separable into thinner fibers and ultimately into fibrils. This habit accounts for greater flexibility and higher tensile strength than other habits of the same mineral. For more information on asbestiform mineralogy, see Refs **(1-3)**.[3]

3.1.2 *asbestos*—a collective term that describes a group of naturally occurring, inorganic, highly fibrous, silicate dominated minerals, which are easily separated into long, thin, flexible fibers when crushed or processed.

3.1.2.1 *Discussion*—Included in the definition are the asbestiform varieties of: serpentine (chrysotile); riebeckite (crocidolite); grunerite (grunerite asbestos); anthophyllite (anthophyllite asbestos); tremolite (tremolite asbestos); and actinolite (actinolite asbestos). The amphibole mineral compositions are defined according to nomenclature of the International Mineralogical Association **(3)**.

Asbestos	Chemical Abstract Service No.[A]
Chrysotile	12001-29-5
Crocidolite	12001-28-4
Grunerite Asbestos	12172-73-5
Anthophyllite Asbestos	77536-67-5
Tremolite Asbestos	77536-68-6
Actinolite Asbestos	77536-66-4

[A] The non-asbestiform variations of the minerals indicated in 3.1.2.1 have different Chemical Abstract Service (CAS) numbers.

3.1.3 *fibril*—a single fiber that cannot be separated into smaller components without losing its fibrous properties or appearance.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *aspect ratio*—the ratio of the length of a fibrous particle to its average width.

3.2.2 *bundle*—a structure composed of three or more fibers in a parallel arrangement with the fibers closer than one fiber diameter to each other.

3.2.3 *cluster*—a structure with fibers in a random arrangement such that all fibers are intermixed and no single fiber is isolated from the group; groupings of fibers must have more than two points touching.

3.2.4 *debris*—materials that are of an amount and size (particles greater than 1 mm in diameter) that can be visually identified as to their source.

3.2.5 *dust*—any material composed of particles in a size range of <1 mm.

3.2.6 *fiber*—a structure having a minimum length of 0.5 µm, an aspect ratio of 5:1 or greater, and substantially parallel sides **(4)**.

3.2.7 *fibrous*—of a mineral composed of parallel, radiating, or interlaced aggregates of fibers, from which the fibers are sometimes separable: that is, the crystalline aggregate may be referred to as fibrous even if it is not composed of separable fibers, but has that distinct appearance.

3.2.7.1 *Discussion*—The term fibrous is used in a general mineralogical way to describe aggregates of grains that crystallize in a needle-like habit and appear to be composed of fibers. Fibrous has a much more general meaning than asbestos. While it is correct that all asbestos minerals are fibrous, not all minerals having fibrous habits are asbestos.

3.2.8 *indirect preparation*—a method in which a sample passes through one or more intermediate steps prior to final filtration.

3.2.9 *matrix*—a structure in which one or more fibers, or fiber bundles that are touching, are attached to, or partially concealed by a single particle or connected group of non-fibrous particles in which the exposed fiber must meet the fiber definition (see 3.2.6).

3.2.10 *structures*—a term that is used to categorize all the types of asbestos particles which are recorded during the analysis (such as fibers, bundles, clusters, and matrices).

3.2.10.1 *Discussion*—Final results of the test are always expressed in asbestos structures per square centimetre.

4. Summary of Test Method

4.1 The sample is collected by vacuuming a known surface area with a standard 25 or 37-mm air sampling cassette using a plastic tube that is attached to the inlet orifice which acts as a nozzle. The sample is transferred from inside the cassette to an aqueous suspension of known volume. Aliquots of the suspension are then filtered through a membrane. A section of the membrane is prepared and transferred to a TEM grid using the direct transfer method. The asbestiform structures are identified, sized, and counted by TEM, using SAED and EDXA at a magnification of 15 000 to 20 000×.

5. Significance and Use

5.1 This microvacuum sampling and indirect analysis method is used for the general testing of non-airborne dust samples for asbestos. It is used to assist in the evaluation of dust that may be found on surfaces in buildings such as ceiling tiles, shelving, electrical components, duct work, carpet, etc. This test method provides an index of the surface loading of asbestos structures in the dust per unit area analyzed as derived from a quantitative TEM analysis.

5.1.1 This test method does not describe procedures or techniques required to evaluate the safety or habitability of buildings with asbestos-containing materials, or compliance with federal, state, or local regulations or statutes. It is the user's responsibility to make these determinations.

5.1.2 At present, no relationship has been established between asbestos-containing dust as measured by this test method and potential human exposure to airborne asbestos. Accordingly, the users should consider other available information in their interpretation of the data obtained from this test method.

5.2 This definition of dust accepts all particles small enough to pass through a 1-mm (No. 18) screen. Thus, a single, large asbestos containing particle(s) (from the large end of the particle size distribution) dispersed during sample preparation may result in anomalously large asbestos surface loading results in the TEM analyses of that sample. It is, therefore,

[3] The boldface numbers in parentheses refer to a list of references at the end of this standard.

recommended that multiple independent samples are secured from the same area, and that a minimum of three samples be analyzed by the entire procedure.

6. Interferences

6.1 The following minerals have properties (that is, chemical or crystalline structure) which are very similar to asbestos minerals and may interfere with the analysis by causing a false positive to be recorded during the test. Therefore, literature references for these materials must be maintained in the laboratory for comparison to asbestos minerals so that they are not misidentified as asbestos minerals.

6.1.1 *Antigorite.*
6.1.2 *Palygorskite (Attapulgite).*
6.1.3 *Halloysite.*
6.1.4 *Pyroxenes.*
6.1.5 *Sepiolite.*
6.1.6 *Vermiculite scrolls.*
6.1.7 *Fibrous talc.*
6.1.8 Hornblende and other amphiboles other than those listed in 3.1.2.

6.2 Collecting any dust particles greater than 1 mm in size in this test method may cause an interference and, therefore, must be avoided.

7. Materials and Equipment

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.[4]

7.2 *Transmission Electron Microscope (TEM),* an 80 to 120 kV TEM, capable of performing electron diffraction, with a fluorescent screen inscribed with calibrated gradations, is required. The TEM must be equipped with energy dispersive X-ray spectroscopy (EDXA) and it must have a scanning transmission electron microscopy (STEM) attachment or be capable of producing a spot size of less than 250 nm in diameter in crossover.

7.3 *Energy Dispersive X-ray System (EDXA).*

7.4 *High Vacuum Carbon Evaporator,* with rotating stage.

7.5 *High Efficiency Particulate Air (HEPA),* filtered negative flow hood.

7.6 *Exhaust or Fume Hood.*

7.7 *Particle-free Water* (ASTM Type II, see Specification D1193).

7.8 *Glass Beakers* (50 mL).

7.9 *Glass Sample Containers,* with wide mouth screw cap (200 mL) or equivalent sealable container (height of the glass sample container should be approximately 13 cm high by 6 cm wide).

7.10 *Waterproof Markers.*

7.11 *Forceps* (tweezers).

7.12 *Ultrasonic Bath,* table top model (100 W).

7.13 *Graduated Pipettes* (1, 5, 10-mL sizes), glass or plastic.

7.14 *Filter Funnel,* either 25 mm or 47 mm, glass or disposable. Filter funnel assemblies, either glass or disposable plastic, and using either a 25-mm or 47-mm diameter filter.

7.15 *Side Arm Filter Flask,* 1000 mL.

7.16 *Mixed Cellulose Ester (MCE) Membrane Filters,* 25 or 47-mm diameter, ≤0.22-µm and 5-µm pore size.

7.17 *Polycarbonate (PC) Filters,* 25 or 47-mm diameter, ≤0.2-µm pore size.

7.18 *Storage Containers,* for the 25 or 47-mm filters (for archiving).

7.19 *Glass Slides,* approximately 76 by 25 mm in size.

7.20 *Scalpel Blades,* No. 10, or equivalent.

7.21 *Cabinet-type Desiccator,* or low temperature drying oven.

7.22 *Chloroform,* reagent grade.

7.23 *Acetone,* reagent grade.

7.24 *Dimethylformamide (DMF).*

7.25 *Glacial Acetic Acid.*

7.26 *1-methyl-2-pyrrolidone.*

7.27 *Plasma Asher,* low temperature.

7.28 *pH Paper.*

7.29 *Air Sampling Pump,* low volume personal-type, capable of achieving a flow rate of 1 to 5 L/min.

7.30 *Rotameter.*

7.31 *Air Sampling Cassettes,* 25 mm or 37 mm, containing 0.8 µm or smaller pore size MCE or PC filters.

7.32 *Cork Borer,* 7 mm.

7.33 *Non-Asbestos Mineral,* references as outlined in 6.1.

7.34 *Asbestos Standards,* as outlined in 3.1.2.

7.35 *Tygon*[5] Tubing, or equivalent.

7.36 *Small Vacuum Pump,* that can maintain a pressure of 92 kPa.

7.37 *Petri Dishes,* large glass, approximately 90 mm in diameter.

7.38 *Jaffe Washer,* stainless steel or aluminum mesh screen, 30 to 40 mesh, and approximately 75 mm by 50 mm in size.

[4] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

[5] Tygon is a registered trademark of the DuPont Co.

7.39 *Copper TEM Finder Grids,* 200 mesh.

7.40 *Carbon Evaporator Rods.*

7.41 *Lens Tissue.*

7.42 *Ashless Filter Paper Filters,* 90-mm diameter.

7.43 *Gummed Paper Reinforcement Rings.*

7.44 *Wash Bottles,* plastic.

7.45 *Reagent Alcohol,* HPLC Grade (Fisher A995 or equivalent).

7.46 *Opening Mesh Screen,* plastic, 1.0 by 1.0 mm, (Spectra-Mesh #146410 or equivalent).

7.47 *Diffraction Grating Replica.*

8. Sampling Procedure for Microvacuum Technique

8.1 For sampling asbestos-containing dust in either indoor or outdoor environments, commercially available cassettes must be used. Air monitoring cassettes containing 25-mm or 37-mm diameter mixed cellulose ester (MCE) or polycarbonate (PC) filter membranes with a pore size less than or equal to 0.8 µm are required (7.31). The number of samples collected depends upon the specific circumstances of the study.

8.2 Maintain a log of all pertinent sampling information and sampling locations.

8.3 Sampling pumps and flow indicators shall be calibrated using a certified standard apparatus or assembly (see Practice D3195 and 7.29).

8.4 Record all calibration information (**5**).

8.5 Perform a leak check of the sampling system at each sampling site by activating the pump (7.29) with the closed sampling cassette in line. Any air flow shows that a leak is present that must be eliminated before initiating the sampling operation.

8.6 Attach the sampling cassette to the sampling pump at the outlet side of the cassette with plastic tubing (7.35). The plastic tubing must be long enough in that the sample areas can be reached without interference from the sampling pump. Attach a clean, approximately 25.4-mm long piece of plastic tubing (6.35-mm internal diameter) directly to the inlet orifice. Use this piece of tubing as the sampling nozzle. Cut the sampling end of the tubing at a 45° angle as illustrated in Fig. 1. The exact design of the nozzle is not critical as long as some vacuum break is provided to avoid simply pushing the dust around on the surface with the nozzle rather than vacuuming it into the cassette. The internal diameter of the nozzle and flow rate of the pump may vary as long as the air velocity is 100 (±10) cm/s. This air velocity calculation is based on an internal sampling tube diameter of 6.35 mm at a flow rate of 2 L/min.

8.7 Measure and determine the sample area of interest. A sample area of 100 cm^2 is vacuumed until there is no visible dust or particulates matter remaining. Perform a minimum of two orthogonal passes on the surface within a minimum of 2 min of sampling time. Avoid scraping or abrading the surface being sampled. (Do not sample any debris or dust particles greater than 1 mm in diameter.) Smaller or larger areas can be sampled, if needed. For example, some surfaces of interest may have a smaller area than 100 cm^2. Less dusty surfaces may require vacuuming of larger areas. Unlike air samples, the overloading of the cassettes with dust will not be a problem. As defined in 3.2.5, only dust shall be collected for this analysis.

8.8 At the end of sample collection, invert the cassette so that the nozzle inlet faces up before shutting off the power to the pump. The nozzle is then sealed with a cassette end-plug and the cassette/nozzle taped or appropriately packaged to prevent separation of the nozzle and cassette assembly. A second option is the removal of the nozzle from the cassette, then plugging of the cassette and shipment of the nozzle (also plugged at both ends) sealed in a separate closeable plastic bag. A third option is placing the nozzle inside the cassette for shipment. The nozzle is always saved and rinsed because a significant percentage of the dust drawn from a lightly loaded surface may adhere to the inside walls of the tubing.

8.9 Check that all samples are clearly labeled, that all dust sampling information sheets are completed, and that all pertinent information has been enclosed, in accordance with laboratory quality control practices, before transfer of the samples to the laboratory. Include an unused cassette and nozzle as a field blank.

8.10 Wipe off the exterior surface of the cassettes with disposable wet towels (baby wipes) prior to packaging for shipment.

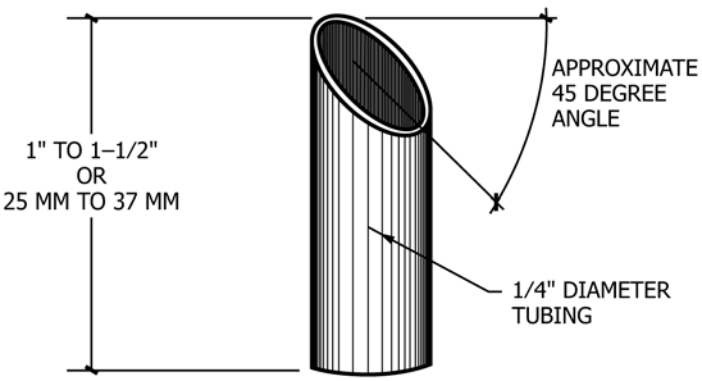

FIG. 1 Example of the Tubing Nozzle

9. Sample Shipment

9.1 Ship dust samples to an analytical laboratory in a sealed container, but separate from any bulk or air samples. The cassettes must be tightly sealed and packed in a material free of fibers or dust to minimize the potential for contamination. Plastic "bubble pack" is probably the most appropriate material for this purpose.

10. Sample Preparation

10.1 Under a negative flow HEPA hood (7.5), carefully wet-wipe the exterior of the cassettes to remove any possible contamination before taking cassettes into a clean preparation area.

10.2 Perform sample preparation in a clean facility that has a separate work area from both the bulk and air sample preparation areas.

10.3 Initial specimen preparation shall take place in a clean HEPA filtered negative pressure hood to avoid any possible contamination of the laboratory or personnel, or both, by the potentially large number of asbestos structures in an asbestos-containing dust sample. Cleanliness of the preparation area hoods is measured by the cumulative process blank surface loadings (see Section 11).

10.4 All sample preparation steps 10.4.1 – 10.4.6 shall take place in the dust preparation area inside a HEPA hood.

10.4.1 Remove the upper plug from the sample cassette and carefully introduce approximately 10-mL solution of a 50/50 mixture of particle-free water and reagent alcohol into the cassette using a plastic wash bottle (7.44). If the plugged nozzle was left attached to the cassette, then remove the plug and introduce the water/alcohol solution into the cassette through the tubing, and then remove the tubing, if it is visibly clean.

10.4.2 Replace the upper plug or the sample cap and lightly shake the dust solution by hand for 3 s.

10.4.3 Remove the entire cap of the cassette and pour the suspension through a 1.0 by 1.0-mm opening screen (7.46) into a pre-cleaned 200-mL glass specimen bottle (7.9). All visible traces of the sample contained in the cassette shall be rinsed through the screen into the specimen bottle with a plastic wash bottle containing the 50/50 solution of particle-free water and alcohol. Repeat this procedure two additional times for a total of three washings. Next, rinse the nozzle two or three times through the screen into the specimen bottle with the 50/50 mixture of water and alcohol. Typically, the total amount of the 50/50 mixture used in the rinse is 50 to 75 mL. Discard the 1.0 by 1.0-mm screen and bring the volume of suspension in the specimen bottle up to the 100-mL mark on the side of the bottle with particle-free water only.

10.4.4 Adjust the pH of the suspension to 3 to 4 using a 10.0 % solution of acetic acid. Use pH paper for testing. Filter the suspension within 24 h to avoid problems associated with bacterial and fungal growth.

10.4.5 Use either a disposable plastic filtration unit or a glass filtering unit (7.14) for filtration of aliquots of the suspension. The ability of an individual filtration unit to produce a uniform distribution may be tested by the filtration of a colored particulate solution such as diluted India ink (solution of carbon black).

10.4.5.1 If a disposable plastic filtration unit is used, then unwrap a new disposable plastic filter funnel unit (either 25 or 47 mm diameter) and remove the tape around the base of the funnel. Remove the funnel and discard the top filter supplied with the apparatus, retaining the coarse polypropylene support pad in place. Assemble the unit with the adapter and a properly sized neoprene stopper, and attach the funnel to the 1000-mL side-arm vacuum flask (7.15). Place a 5.0-µm pore size MCE (backing filter) on the support pad. Wet it with a few mL of particle-free water and place an MCE (7.16) or PC filter (≤0.22-µm pore size) (7.17) on top of the backing filter. Apply a vacuum (7.36), ensuring that the filters are centered and pulled flat without air bubbles. Any irregularities on the filter surface requires the discard of that filter. After the filter has been seated properly, replace the funnel and reseal it with the tape. Return the flask to atmospheric pressure.

10.4.5.2 If a glass filtration unit is used, place a 5-µm pore size MCE (backing filter) on the glass frit surface. Wet the filter with particle-free water, and place an MCE or PC filter (≤0.22-µm pore size) on top of the backing filter. Apply a vacuum, ensuring that the filters are centered and pulled flat without air bubbles. Replace the filters if any irregularities are seen on the filter surface. Before filtration of each set of sample aliquots, prepare a blank filter by filtration of 50 mL of particle-free water. If aliquots of the same sample are filtered in order of increasing surface loading, the glass filtration unit need not be washed between filtration. After completion of the filtration, do not allow the filtration funnel assembly to dry because contamination is then more difficult to remove. Wash any residual solution from the filtration assembly by holding it under a flow of water, then rub the surface with a clean paper towel soaked in a detergent solution. Repeat the cleaning operation, and then rinse two times in particle-free water.

10.4.6 With the flask at atmospheric pressure, add 20 mL of particle-free water into the funnel. Cover the filter funnel with its plastic cover if the disposable filtering unit is used.

10.4.7 Briefly hand shake (3 s) the capped bottle with the sample suspension, then place it in a tabletop ultrasonic bath (7.12) and sonicate for 3.0 min. Maintain the water level in the sonicator at the same height as the suspension in sample bottle. The ultrasonic bath shall be calibrated as described in 20.5. The ultrasonic bath must be operated at equilibrium temperature. After sonicating, return the sample bottle to the work surface of the HEPA hood. Preparation steps 10.4.8 – 10.4.14 shall be carried out in this hood.

10.4.8 Shake the suspension lightly by hand for 3 s, then let it rest for 2.0 min to allow large particles to settle to the bottom of the bottle or float to the surface.

10.4.9 Estimate the amount of liquid to be withdrawn to produce an adequate filter preparation. Experience has shown that a light staining of the filter surface will yield a suitable preparation for analysis. Filter at least 1.0 mL, but no more than half the total volume. If after examination in the TEM, the smallest volume measured (1.0 mL) (7.13) yields an over-loaded sample, then perform additional serial dilutions of the

suspension. If it is estimated that less than 1.0 mL of suspension has to be filtered because of the density of the suspension, perform a serial dilution.

10.4.9.1 If serial dilutions are required, repeat step 10.4.8 before the serial dilution portion is taken. Do not re-sonicate the original suspension or any serial dilutions. The recommended procedure for a serial dilution is to mix 10 mL of the sample suspension with 90 mL of particle-free water in a clean sample bottle to obtain a 1:10 serial dilution. Follow good laboratory practices when performing dilutions.

10.4.10 Insert a new disposable pipette halfway into the sample suspension and withdraw a portion. Avoid pipetting any of the large floating or settled particles. Uncover the filter funnel and dispense the mixture from the pipette into the water in the funnel.

10.4.11 Apply vacuum to the flask and draw the mixture through the filter.

10.4.12 Discard the pipette.

10.4.13 Disassemble the filtering unit and carefully remove the sample filter with fine tweezers (7.11). Place the completed sample filter particle side up, into a precleaned, labeled, disposable, plastic petri dish) or other similar container.

10.4.14 In order to ensure that an optimally-loaded filter is obtained, it is recommended that filters be prepared from several different aliquots of the dust suspension. For this series of filters, it is recommended that the volume of each aliquot of the original suspension be a factor of five higher than the previous one. If the filters are prepared in order of increasing aliquot volume, all of the filters for one sample can be prepared using one plastic disposable filtration unit, or without cleaning of glass filtration equipment between individual filtration. Before withdrawal of each aliquot from the sample, shake the suspension without additional sonification and allow to rest for 2 min.

10.4.15 There are many practical methods for drying MCE filters. The following are two examples that can be used: (*1*) dry MCE filters for at least 12 h (over desiccant) in an airtight cabinet-type desiccator (7.21); (*2*) to shorten the drying time (if desired), remove a plug of the damp filter and attach it to a glass slide (7.19) as described in 12.1.2 and 12.1.3. Place the slide with a filter plug or filter plugs (up to eight plugs can be attached to one slide) on a bed of desiccant, in the desiccator for 1 h.

10.4.16 PC filters do not require lengthy drying before preparation, but shall be placed in a desiccator for at least 30 min before preparation.

10.5 Prepare TEM specimens from small sections of each dried filter using the appropriate direct transfer preparation method.

11. Blanks

11.1 Prepare sample blanks that include both a process blank (50 mL of particle-free water) for each set of samples analyzed and one unused filter from each new box of sample filters (MCE or PC) used in the laboratory. If glass filtering units are used, prepare and analyze a process blank each time the filtering unit is cleaned. Blanks will be considered contaminated, if after analysis, they are shown to contain more than 53 asbestos structures per square millimetre. This generally corresponds to three or four asbestos structures found in ten grid openings. The source of the contamination must be found before any further analysis can be performed. Reject samples that were processed along with the contaminated blanks and prepare new samples after the source of the contamination is found.

11.2 Prepare field blanks which are included with sample sets in the same manner as the samples, to test for contamination during the sampling, shipping, handling, and preparation steps of the method.

12. TEM Specimen Preparation of Mixed Cellulose Ester (MCE) Filters

NOTE 1—Use of either the acetone or the diamethylformamide-acetic acid method is acceptable.

12.1 *Acetone Fusing Method:*

12.1.1 Remove a section (a plug) from any quadrant of the sample and blank filters. Sections can be removed from the filters using a 7-mm cork borer (7.32). The cork borer must be wet wiped after each time a section is removed.

12.1.2 Place the filter section (particle side up) on a clean microscope slide. Affix the filter section to the slide with a gummed page reinforcement (7.43), or other suitable means. Label the slide with a glass scribing tool or permanent marker (7.10).

12.1.3 Prepare a fusing dish from a glass petri dish (7.37) and a metal screen bridge (7.38) with a pad of five to six ashless paper filters (7.42) and place in the bottom of the petri dish (**4**). Place the screen bridge on top of the pad and saturate the filter pads with acetone. Place the slide on top of the bridge in the petri dish and cover the dish. Wait approximately 5 min for the sample filter to fuse and clear.

12.2 *Dimethylformamide-Acetic Acid Method:*

12.2.1 Place a drop of clearing solution that consists of 35 % dimethylformamide (DMF), 15 % glacial acetic acid, and 50 % Type II water (v/v) on a clean microscope slide. Gauge the amount used so that the clearing solution just saturates the filter section.

12.2.2 Carefully lay the filter segment, sample surface upward, on top of the solution. Bring the filter and solution together at an angle of about 20° to help exclude air bubbles. Remove any excess clearing solution. Place the slide in an oven or on a hot plate, in a fume hood, at 65 to 70°C for 10 min.

12.3 Plasma etching of the collapsed filter is required.

12.3.1 The microscope slide to which the collapsed filter pieces are attached is placed in a plasma asher (7.27). Because plasma ashers vary greatly in their performance, both from unit to unit and between different positions in the asher chamber, it is difficult to specify the exact conditions that must be used. Insufficient etching will result in a failure to expose embedded fibers, and too much etching may result in the loss of particles from the filter surface. To determine the optimum time for ashing, place an unused 25 mm diameter MCE filter in the center of a glass microscope slide. Position the slide approximately in the center of the asher chamber. Close the chamber and evacuate to a pressure of approximately 40 Pa, while

admitting oxygen to the chamber at a rate of 8 to 20 cm^3/min. Adjust the tuning of the system so that the intensity of the plasma is maximized. Determine the time required for complete oxidation of the filter. Adjust the system parameters to achieve complete oxidation of the filter in a period of approximately 15 min. For etching of collapsed filters, use these operating parameters for a period of 8 min. For additional information on calibration, see the *USEPA Asbestos-Containing Materials in Schools* (**4**) or *NIST/NVLAP Program Handbook for Airborne Asbestos Analysis*(**6**) documents.

12.3.2 Place the glass slide containing the collapsed filters into the low-temperature plasma asher, and etch the filter.

12.4 Carbon coating of the collapsed and etched filters is required.

12.4.1 Carbon coating must be performed with a high-vacuum coating unit (7.4), capable of less than 10^{-4} torr (13 MPa) pressure. Units that are based on evaporation of carbon filaments in a vacuum generated only by an oil rotary pump have not been evaluated for this application and shall not be used. Carbon rods (7.40) used for evaporators shall be sharpened with a carbon rod sharpener to a neck of about 4 mm in length and 1 mm in diameter. The rods are installed in the evaporator in such a manner that the points are approximately 100 to 120 mm from the surface of the microscope slide held in the rotating device.

12.4.2 Place the glass slide holding the filters on the rotation device, and evacuate the evaporator chamber to a vacuum of at least 13 MPa. Perform the evaporation in very short bursts, separated by 3 to 4 s to allow the electrodes to cool. An alternate method of evaporation is by using a slow continuous applied current. An experienced analyst can judge the thickness of the carbon film to be applied. Conduct tests on unused filters first. If the carbon film is too thin, large particles will be lost from the TEM specimen, and there will be few complete and undamaged grid openings on the specimen.

12.4.2.1 If the coating is too thick, it will lead to a TEM image that is lacking in contrast, and the ability to obtain electron diffraction patterns will be compromised. The carbon film shall be as thin as possible and still remain intact on most of the grid openings of the TEM specimen.

12.5 *Preparation of the Jaffe Washer*—The precise design of the Jaffe washer is not considered important, so any one of the published designs may be used (**7**, **8**). One such washer consists of a simple stainless steel bridge contained in a glass petri dish.

12.5.1 Place several pieces of lens tissue (7.41) on the stainless steel bridge. The pieces of lens tissue shall be large enough to completely drape over the bridge and into the solvent. In a fume hood, fill the petri dish with acetone (or DMF) until the height of the solvent is brought up to contact the underside of the metal bridge as illustrated in Fig. 2.

12.6 *Placing the Specimens into the Jaffe Washer:*

12.6.1 Place the TEM grids (7.39) shiny side up on a piece of lens tissue or filter paper so that individual grids can be easily picked up with tweezers.

12.6.2 Prepare three grids from each sample.

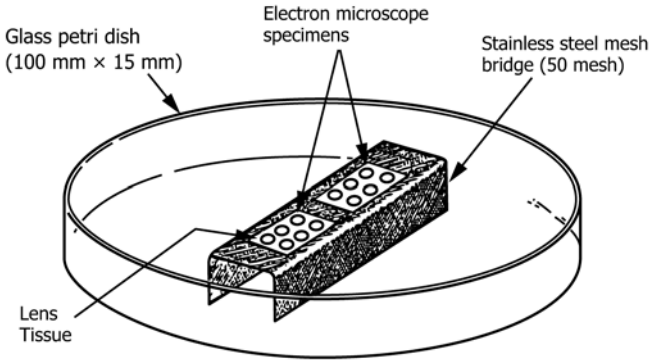

FIG. 2 Example of Design of Solvent Washer (Jaffe Washer)

12.6.2.1 Using a curved scalpel blade (7.20), excise at least two square (3 mm by 3 mm) pieces of the carbon-coated MCE filter from the glass slide.

12.6.2.2 Place the square filter piece carbon-side up on top of a TEM specimen grid.

12.6.2.3 Place the whole assembly (filter/grid) on the saturated lens tissue in the Jaffe washer.

12.6.2.4 Place the three TEM grid sample filter preparations on the same piece of lens tissue in the Jaffe washer.

12.6.2.5 Place the lid on the Jaffe washer and allow the system to stand for several hours.

12.7 Alternately, place the grids on a low level (petri dish filled to the ⅛ mark) DMF Jaffe washer for 60 min. Add enough solution of equal parts DMF/acetone to fill the washer to the screen level. Remove the grids after 30 min if they have cleared, that is, all filter material has been removed from the carbon film, as determined by inspection in the TEM.

12.8 Carefully remove the grids from the Jaffe washer, allowing the grids to dry before placing them in a clean marked grid box.

13. TEM Specimen Preparation of Polycarbonate (PC) Filter

13.1 Cover the surface of a clean microscope slide with two strips of double-sided adhesive tape.

13.2 Cut a strip of filter paper slightly narrower than the width of the slide. Position the filter paper strip on the center of the length of the slide.

13.3 Using a clean, curved scalpel blade, cut a strip of the PC filter approximately 25 by 6 mm. Use a rocking motion of the scalpel blade to avoid tearing the filter. Place the PC strip particle side up on the slide perpendicular to the long axis of the slide. The ends of the PC strip must contact the double sided adhesive tape. Each slide can hold several PC strips. With a glass marker, label each PC strip with the individual sample number.

13.4 Carbon coat the PC filter strips as discussed in 12.4.2. PC filters do not require etching. (**Warning—**Do not overheat the filter sections while carbon coating.)

13.5 Prepare a Jaffe washer as described in 12.5, but fill the washer with chloroform or 1-methyl-2-pyrrolidone to the level of the screen.

13.6 Using a clean curved scalpel blade, excise three, 3-mm square filter pieces from each PC strip. Place the filter squares carbon side up on the shiny side of a TEM grid. Pick up the grid and filter section together and place them on the lens tissue in the Jaffe washer.

13.7 Place the lid on the Jaffe washer and rest the grids in place for at least 4 h. Best results are obtained with longer wicking times, up to 12 h.

13.8 Carefully remove the grids from the Jaffe washer, allowing the grids to dry before placing them in a clean, marked grid box.

14. Grid Opening Measurements

14.1 TEM grids must have a known grid opening area. Determine this area as follows:

14.2 Measure at least 20 grid openings in each of 20 random 75 to 100 µm (200-mesh) copper grids for a total of 400 grid openings for every 1000 grids used, by placing the 20 grids on a glass slide and examining them under the optical microscope. Use a calibrated graticule to measure the average length and width of the 20 openings from each of the individual grids. From the accumulated data, calculate the average grid opening area of the 400 openings.

14.3 Grid area measurements can also be made at the TEM at a calibrated screen magnification of between 15 000 and 20 000×. Typically measure one grid opening for each grid examined. Measure grid openings in both the x and y directions and calculate the area.

14.4 Pre-calibrated TEM grids are also acceptable for this test method.

15. TEM Method

15.1 Microscope settings: 80 to 120 kV, 15 000 to 20 000× screen magnification for analysis (7.2).

15.2 Analyze two grids for each sample. Analyze one-half of the sample area on one sample grid preparation and the remaining half on a second sample grid preparation.

15.3 *Determination of Specimen Suitability:*

15.3.1 Carefully load the TEM grid, carbon side facing up (in the TEM column) with the grid bars oriented parallel/perpendicular to the length of the specimen holder. Use a hand lens or loupe, if necessary. This procedure will line up the grid with the *x* and *y* translation directions of the microscope. Insert the specimen holder into the microscope.

15.3.2 Scan the entire grid at low magnification (250 to 1000×) to determine its suitability for high magnification analysis as specified in 15.3.3.

15.3.3 Grids are acceptable for analysis if the following conditions are met:

15.3.3.1 The fraction of grid openings covered by the replica section is at least 50 %.

15.3.3.2 Relative to that section of the grid covered by the carbon replica, the fraction of intact grid openings is greater than 50 %.

15.3.3.3 The fractional area of undissolved filter is less than 10 %.

15.3.3.4 The fraction of grid openings with overlapping or folded replica film is less than 50 %.

15.3.3.5 At least 20 grid openings, that have no overlapping or folded replica, are less than 5 % covered with holes and have less than 5 % opaque area due to incomplete filter dissolution.

15.4 *Determination of Grid Opening Suitability:*

15.4.1 If the grid meets acceptance criteria, choose a grid opening for analysis from various areas of the grid so that the entire grid is represented. Determine the suitability of each individual grid opening prior to the analysis.

15.4.2 The individual grid opening must have less than 5 % holes over its area.

15.4.3 Grid openings must be less than 25 % covered with particulate matter.

15.4.4 Grid openings must be uniformly loaded.

15.5 Observe and record the orientation of the grid at 80 to 150×, on a grid map record sheet along with the location of the grid openings that are examined for the analysis. If indexed grids are used, a grid map is not required, but the identifying coordinates of the grid square must be recorded.

16. Recording Data Rules

16.1 Record on the count sheet any continuous grouping of particles in which an asbestos fiber is detected. Classify asbestos structures as fibers, bundles, clusters, or matrices as defined in 5.2.

16.2 Use the criteria for fiber, bundle, cluster, and matrix identification, as described in the *USEPA Asbestos-ContainingMaterials in Schools* document (**4**). Record, for each AHERA structure identified, the length and width measurements.

16.3 Record NSD (No Structures Detected) when no structures are detected in the grid opening.

16.4 Identify structures classified as chrysotile identified by either electron diffraction or X-ray analysis (7.3) and recorded on a count sheet. Verify at least one out of every ten chrysotile structures by X-ray analysis.

16.5 Structures classified as amphiboles by X-ray analysis and electron diffraction are recorded on the count sheet. For more information on identification, see Yamate, et al., (**7**) or Chatfield and Dillon (**8**).

16.6 Record a typical electron diffraction pattern for each type of asbestos observed for each group of samples (or a minimum of every five samples) analyzed. Record the micrograph number on the count sheet. Record at least one X-ray spectrum for each type of asbestos observed per sample. Attach the print-outs to the back of the count sheet. If the X-ray spectrum is stored, record the file and disk number on the count sheet.

16.7 *Counting Rules:*

16.7.1 At a screen magnification of between 15 000 and 20 000× evaluate the grids for the most concentrated sample loading; reject the sample if it is estimated to contain more than 50 asbestos structures per grid opening. Proceed to the next lower concentrated sample until a set of grids are obtained that have less than 30 asbestos structures per grid opening.

16.8 *Analytical Sensitivity (AS)*—As determined by the following equation:

$$(EFA \times 100\,mL \times 1)/(GO \times GOA \times V \times SPL) = AS \quad (1)$$

where:

EFA = effective filter area of the final sampling filter, mm^2,
GO = number of grid openings counted,
GOA = average grid opening area, mm^2,
SPL = surface area sampled, cm^2,
V = volume of sample filtered in step 10.4.9, representing the actual volume taken from the original 100-mL suspension, mL, and
AS = analytical sensitivity, expressed as asbestos structures/cm^2.

16.8.1 An AS of approximately 1000 asbestos structures per square centimetre (calculated for the detection of a single asbestos structure) has been designed for this analysis. This sensitivity can be achieved by increasing the amount of liquid filtered, increasing the number of grid openings analyzed, increasing the area of the collection, or decreasing the size of the final filter. For example, using a collection area = 500 cm^2, filter area = 1000 mm^2, number of grid openings = 10, and a grid area of 0.01 mm^2, V = 50 mL, the AS is 40 str/cm^2. Occasionally, due to high particle loadings or high asbestos surface loading, this AS cannot be practically achieved and stopping rules apply.

16.8.2 The numerical value of AS required for any specific application of this method may be achieved by selecting an appropriate combination of the sampling and analysis parameters in the above equation. For example, if SPL = 100 cm^2, EFA = 1000 mm^2, GO = 10, GOA = 0.01 mm^2, V = 10 mL, and D = 1, then AS = 1000 str/cm^2. Increasing GO to 50 and V to 50 mL changes the AS to 40 Str/cm^2.

16.8.3 When sample filters are heavily loaded with particulate matter, it may useful to employ serial dilutions during preparation to reduce the loading on grid specimens to acceptable levels and thus facilitate analysis. Under such circumstances, the AS may be calculated by substituting an appropriate value for the dilution factor, D, into the above equation. In general:

$$D = VA/(V + VPFW) \quad (2)$$

where:

VA = the volume of the aliquot from the new, diluted suspension that is filtered to prepare the analytical filter,
V = the volume of the aliquot from the initial (100 mL) suspension that is diluted, and
VPFW = the volume of particle free water added to V during serial dilution to produce the new, diluted suspension.

Thus, if GO = 10, V = 10 mL, VPFW = 90 mL, and VA = 1.0 mL, D = 0.01 and the AS = 100 000 str/cm^2.

16.9 *Limit of Detection*—The limit of detection for this test method is calculated using the Practice D6620. All data shall be provided in the laboratory report.

16.10 *Stopping Rules:*

16.10.1 The analysis is stopped upon the completion of the grid square that achieves an AS of less than 1000 asbestos structures per square centimetre.

16.10.2 If an AS of 1000 asbestos structures per square centimetre cannot be achieved after analyzing ten grid openings then stop on grid opening No. 10 or the grid opening which contains the 100th asbestos structure, whichever comes first. A minimum of four grid squares shall be analyzed for each sample.

16.10.2.1 If the analysis is stopped because of the 100th structure rule, the entire grid square containing the 100th structure must be counted.

16.11 After analysis, remove the grids from the TEM, and replace them in the appropriate grid storage holder.

17. Sample Storage

17.1 The washed-out sample cassettes can be discarded after use.

17.2 Sample grids and unused filter sections (7.18) must be stored for a minimum of one year.

18. Reporting

18.1 Report the following information for each dust sample analyzed:

18.1.1 Surface loading in structures/cm^2.
18.1.2 The AS.
18.1.3 Types of asbestos present.
18.1.4 Number of asbestos structures counted.
18.1.5 Effective filtration area.
18.1.6 Average size of the TEM grid openings that were counted.
18.1.7 Number of grid openings examined.
18.1.8 Sample dilution used.
18.1.9 Area of the surface sampled.
18.1.10 Listing of size data for each structure counted.
18.1.11 A copy of the TEM count sheet or a complete listing of the raw data. An example of a typical count sheet is shown in Appendix X1.

18.2 Determine the amount of asbestos in any accepted sample using the following formula:

$$\frac{EFA \times 100\ mL \times \#STR}{GO \times GOA \times V \times SPL} = \text{asbestos structures/cm}^2 \quad (3)$$

where:

 # STR = number of asbestos structures counted,
EFA = effective filter area of the final sampling filter, mm^2,
GO = number of grid openings counted,
GOA = average grid opening area, mm^2,
SPL = surface area sampled, cm^2, and
V = volume of sample filtered in step 10.4.9, representing the actual volume taken from the original 100 mL suspension, mL.

19. Quality Control/Quality Assurance

19.1 In general, the laboratory's quality control checks are used to verify that a system is performing according to

specifications regarding accuracy and consistency. In an analytical laboratory, spiked or known quantitative samples are normally used. However, due to the difficulties in preparing known quantitative asbestos samples, routine quality control testing focuses on re-analysis of samples (duplicate recounts).

19.1.1 Re-analyze samples at a rate of 1/10 of the sample sets (one out of every ten samples analyzed not including laboratory blanks). The re-analysis shall consist of a second sample preparation obtained from the final filter.

19.2 In addition, quality assurance programs must follow the criteria shown in the *USEPA Asbestos-Containing Materials in Schools* document (**4**) and in the *NIST/NVLAP Program Handbook for Airborne Asbestos Analysis* document (**6**). These documents describe sample custody, sample preparation, blank checks for contamination, calibration, sample analysis, analyst qualifications, and technical facilities.

20. Calibrations

20.1 Perform calibrations of the instrumentation on a regular basis, and retain these records in the laboratory, in accordance with the laboratory's quality assurance program.

20.2 Record calibrations in a log book along with dates of calibration and the attached backup documentation.

20.3 A calibration list for the instrument is as follows:

20.3.1 *TEM:*

20.3.1.1 Check the alignment and the systems operation. Refer to the TEM manufacturer's operational manual for detailed instructions.

20.3.1.2 Calibrate the camera length of the TEM in electron diffraction (ED) operating mode before ED patterns of unknown samples are observed. Camera length can be measured by using a carbon coated grid on which a thin film of gold has been sputtered or evaporated. A thin film of gold is evaporated on the specimen TEM grid to obtain zone-axis ED patterns superimposed with a ring pattern from the polycrystalline gold film. In practice, it is desirable to optimize the thickness of the gold film so that only one or two sharp rings are obtained on the superimposed ED pattern. Thick gold films will tend to mask weak diffraction spots from the fibrous particles. Since the unknown d-spacings of most interest in asbestos analysis are those which lie closest to the transmitted beam, multiple gold rings from thick films are unnecessary. Alternatively, a gold standard specimen can be used to obtain an average camera constant calculated for that particular instrument and can then be used for ED patterns of unknowns taken during the corresponding period.

20.3.1.3 Perform magnification calibration at the fluorescent screen. This calibration must be performed at the magnification used for structure counting. Calibration is performed with a grating replica (7.47) (for example, one containing at least 2160 lines/mm).

(a) Define a field of view on the fluorescent screen. The field of view must be measurable or previously inscribed with a scale or concentric circles (all scales should be metric).

(b) Frequency of calibration will depend on the service history of the particular microscope.

(c) Check the calibration after any maintenance of the microscope that involves adjustment of the power supply to the lens or the high voltage system or the mechanical disassembly of the electron optical column (apart from filament exchange).

(d) The analyst must ensure that the grating replica is placed at the same distance from the objective lens as the specimen.

(e) For instruments that incorporate a eucentric tilting specimen stage, all specimens and the grating replica must be placed at the eucentric position.

20.3.1.4 The smallest spot size of the TEM must be checked.

(a) At the crossover point, photograph the spot size at a screen magnification of 15 000 to 20 000×. An exposure time of 1 s is usually adequate.

(b) The measured spot size must be less than or equal to 250 nm.

20.4 *EDXA:*

20.4.1 The resolution and calibration of the EDXA must be verified.

20.4.1.1 Collect a standard EDXA Cu peak from the Cu grid.

20.4.1.2 Compare the X-ray energy versus channel number for the Cu peak and be certain that readings are within ±10 eV.

20.4.2 Collect a standard EDXA of crocidolite asbestos (NIST SRM 1866).

20.4.2.1 The elemental analysis of the crocidolite must resolve the Na peak.

20.4.3 Collect a standard EDXA of chrysotile asbestos.

20.4.3.1 The elemental analysis of chrysotile must resolve both Si and Mg on a single chrysotile fiber.

20.5 Ultrasonic bath calibration shall be performed as follows:

20.5.1 Fill the bath water to a level equal to the height of suspension in the glass sample container that will be used for the dust analysis. Operate the bath until the water reaches the equilibrium temperature.

20.5.2 Place 100 mL of water (at approximately 20°C) in another 200-mL glass sample container, and record its temperature.

20.5.3 Place the sample container in the water in the ultrasonic bath (with the power turned off). After 60 s, remove the glass container and record its temperature.

20.5.4 Place 100 mL of water (at approximately 20°C) in another 200-mL glass sample container, and record its temperature.

20.5.5 Place the second sample container into the water in the ultrasonic bath (with the power turned on). After 60 s, remove the glass container and record its temperature.

20.5.6 Calculate the rate of energy deposition into the sample container using the following formula:

$$R = 4.185 \times \sigma \times \rho \times \frac{(\theta_2 - \theta_1)}{t} \quad (4)$$

where:

4.185 = Joules/cal,
R = energy deposition, watts/mL,

θ_1 = temperature rise with the ultrasonic bath not operating, °C,
θ_2 = temperature rise with the ultrasonic bath operating, °C,
t = time in seconds, 60 s (20.5.3 and 20.5.5),
σ = specific heat of the liquid in the glass sample container, 1.0 cal/g, and
ρ = density of the liquid in the glass sample container, 1.0 g/cm^3.

20.5.7 Adjust the operating conditions of the bath so that the rate of energy deposition is in the range of 0.08 to 0.12 MW/m^3, as defined by this procedure.

21. Precision and Bias[6]

21.1 *Precision*—The precision of this test method is based on an interlaboratory study conducted in 2003 following the guidance provided in Guide D3670. Each of the ten laboratories tested a single material. Every "test result" represents an individual determination. Each laboratory reported duplicate test results for the analyses. Practice E691 was followed for the design and analysis of the data.

21.1.1 *Repeatability Limit (r)*—Two test results obtained within one laboratory shall be judged not equivalent if they differ by more than the *r* value for that material; *r* is the interval representing the critical difference between two test results for the same material, obtained by the same operator using the same equipment on the same day in the same laboratory.

21.1.1.1 Repeatability limits are listed in Table 1.

21.1.2 *Reproducibility (R)*—Two test results shall be judged not equivalent if they differ by more than the *R* value for that material; *R* is the interval representing the critical difference between two test results for the same material, obtained by different operators using different equipment in different laboratories.

21.1.2.1 Reproducibility limits are listed in Table 1.

21.1.3 The above terms (repeatability limit and reproducibility limit) are used as specified in Practice E177.

21.1.4 Any judgment in accordance with statements 21.1.1 and 21.1.2 would have an approximate 95 % probability of being correct.

21.2 *Bias*—At the time of the study, there was no accepted reference material suitable for determining the bias for this test method, therefore no statement on bias is being made.

21.3 The precision statement was determined through statistical examination of 20 results, from ten laboratories, on a single type of material, described below.

21.3.1 *Material A*—A chrysotile asbestos fiber-containing dust in a microvacuum cassette. The dust cassettes were prepared by resuspending a sample of World Trade Center dust and allowing it to settle. Samples of the dust from 100-cm^2 areas were collected using a microvacuum cassette following the procedures described in this test method.

TABLE 1 Asbestos Structures per cm^2 (×1000)

Material	Average[A] $\bar{x}$	Repeatability Standard Deviation S_r	Reproducibility Standard Deviation S_R	Repeatability Limits *r*	Reproducibility Limits *R*
A	147.80	22.07	85.46	61.80	239.30

[A] The average of the laboratories' calculated averages.

[6] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D22-1032. Contact ASTM Customer Service at service@astm.org.

22. Keywords

22.1 asbestos; microvacuuming; settled dust; TEM

APPENDIX

(Nonmandatory Information)

X1. DUST SAMPLE ANALYSIS

X1.1 See Figs. X1.1 and X1.2 for the dust analysis worksheet and the TEM count sheet.

DUST SAMPLE ANALYSIS

Client: _____
Sample ID: _____
Job Number: _____
Date Sample Analyzed: – –
Number of Openings/Grids Counted: | |
Grid Accepted, 600X: Yes No
Percent Loading: _____ %
Grid Box #1: _____

Accelerating Voltage: _____
Indicated Mag: _____ KX
Screen Mag: _____ KX
Microscope: 1 2 3 4 5
Filter Type: _____
Filter Size: _____
Filter Pore Size (μm): _____
Grid Opening: 1) _____ μm × _____ μm
2) _____ μm × _____ μm

Analyst: _____

Reviewer: _____

Counting Rules: AHERA LEVEL II

Calculation Data:

Effective Filter Area in mm^2:	(EFA)	_____
Number of Grid Openings Counted:	(GO)	_____
Average Grid Opening Area in mm^2:	(GOA)	_____
Volume of sample Filtered in ml:	(V)	_____
Surface area Sampled in cm^2:	(SPL)	_____
Number of Asbestos Structures Counted:*	(#STR)	_____

* If the number of asbestos structures counted is less than or equal to 4, enter 4 structures as the limit of detection here.

FORMULA FOR CALCULATION OF ASBESTOS STRUCTURES "DUST" PER CM^2:

$$\frac{EFAX \times 100 \times \#STR}{GO \times GOA \times V \times SPL} = \text{(Asbestos Structures per } cm^2\text{)}$$

Results for Total Asbestos Structures: _____
(Structures per cm^2)

Results for Structures ≥ microns: _____
(Structures per cm^2)

FIG. X1.1 Dust Sample Analysis Work Sheet

Job Number: _____

Structure #	Grid # Square #	Type	Structure	Length Microns	Width Microns	Confirmation		
						Morph.	SAED	EDS

Note: Keys to Abbreviations Used in Figure:

Type:

C	=	Chrysotile
AM	=	Amosite
CR	=	Crocidolite
AC	=	Actinolite
TR	=	Tremolite
AN	=	Anthophyllite
N	=	Non Asbestos

Structure:

F	=	Fiber
B	=	Bundle
C	=	Cluster
M	=	Matrix

Others:

NSD	=	No Structures Detected
Morph	=	Morphology
SAED	=	Selected Area Electron Diffraction
EDS	=	Energy Dispersive X-Ray Spectroscopy
ER	=	Inter-Row Spacing
NP	=	No Pattern

FIG. X1.2 TEM Count Sheet

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Exhibit 12

Johnson & Johnson
BABY PRODUCTS COMPANY

S 'DARD TEST METHOD

SUBJECT: ANALYSIS OF POWDERED TALC FOR ASBESTIFORM MINERALS BY TRANSMISSION ELECTRON MICROSCOPY

SUPERSEDES: ADL 1305 DATE: 3/8/89 AUTHORIZATION NO.: BCR 011362

1. SCOPE & PURPOSE

This method is applicable to the identification and quantitation of small (typically 1-20 micrometer) asbestiform minerals in powdered talc. Samples may be previously screened with light microscopy or x-ray diffraction techniques.

2. PRINCIPLE OF METHOD

The combined techniques of transmission electron microscopy (TEM), selected area electron diffraction (SAED) and energy dispersive x-ray analysis (EDXRA) permit the detection of asbestiform minerals based on morphological characteristics, followed by a definitive mineralogical identification of each fiber.

3. INTERFERENCES

Interferences are caused by fibrous particles which must be distinguished from positively identifiable asbestos, and by large particles or particle aggregates which may obscure fibers. Positively identified non-asbestos fibers include rolled talc, ribbon talc, antigorite, silica fibers and iron oxide fibers. Organic additives such as perfumes may crystallize out as fibers or needle-shaped crystals in finished cosmetic products. In the absence of positive identification, all other fibers must be classified as unidentifiable.

4. INSTRUMENTAL CONDITIONS

The talc specimen grids are examined in the TEM at an accelerating voltage of 120 kv and at magnification of 20,000X and 5,000X.

5. SENSITIVITY

This method is capable of detecting a single fiber as small as 1 micrometer (μm) long by 0.075 μm wide in the entire TEM field, which results in a theoretical detection limit of 10^{-5} weight percent. Such fibers usually can be identified readily by SAED and EDXRA. The mass of a fiber with the above dimensions is 1.1 x 10^{-14} g for chrysotile and 1.5 x 10^{-14} g for amphibole.

Johnson & Johnson
BABY PRODUCTS COMPANY

STANDARD TEST METHOD

SUBJECT: ANALYSIS OF POWDERED TALC FOR ASBESTIFORM MINERALS BY TRANSMISSION ELECTRON MICROSCOPY

SUPERSEDES: ADL 1305 DATE: 3/8/89 AUTHORIZATION NO.: BCR 011362

6. LIMIT OF QUANTIFIABLE DETECTION

The detection of five or more asbestiform minerals of one variety in an analysis constitutes a quantifiable level of detection. When no asbestiform minerals are detected, a representative fiber size is used to calculate a detection limit. A representative fiber size is 3 μm long by 0.2 μm wide by 0.06 μm thick, which is considerably larger than the smallest fiber that can be detected (see section 5, SENSITIVITY), but is more typical of small asbestos fibers that are detected in talc analyses. The mass of five such fibers is calculated as follows:

3 μm x 0.2 μm x 0.06 μm = 0.036μm³ per fiber
x 3.3E-12 g / μm³ = 1.2 E-13 g per fiber
x 5 fibers = 6E-13 grams per 5 fibers.

The limit of quantifiable detection for most talc analyses is approximately 6 x 10^{-4} weight percent. The theoretical and quantifiable detection limits assume homogeneity of the material being sampled.

QUALITY ASSURANCE

Blank suspensions are routinely prepared and tested in order to monitor potential residual contamination from the sample jars. Blank carbon-coated grids are routinely tested to monitor the ambient fiber count. If greater than 4 fibers per grid are present, the jars are pre-cleaned or new carbon-coated grids are prepared, respective of the test.

8. BACKGROUND CORRECTION

As of the time of this writing, background correction has not been necessary. The amount of background asbestos detected has been insignificant in comparison to the levels of asbestos found in contaminated samples.

9. PREPARATION AND ANALYSIS TIME

Preparation time per sample (including preparation of related materials) is one hour. Analysis search time per sample is a maximum of two hours.

10. APPARATUS

A. Analytical balance with 0.0001 gram sensitivity

B. Weighing boats

C. Narrow spatula

Johnson & Johnson BABY PRODUCTS COMPANY

S DARD TEST METHOD

SUBJECT: ANALYSIS OF POWDERED TALC FOR ASBESTIFORM MINERALS BY TRANSMISSION ELECTRON MICROSCOPY

SUPERSEDES: ADL 1305 DATE: 3/8/89 AUTHORIZATION NO.: BCR 011362

D. Wide mouth polyethylene jars (125 ml)

E. Mild ultrasonic bath, minimum 50 watts

F. Micropipettor (5-10 µl range) with disposable tips

G. Standard 3 mm diameter, 200 mesh, copper TEM grids, covered with a carbon-coated formvar film.

H. Transmission electron microscope (TEM) with an 80-120 kv accelerating voltage and energy dispersive x-ray analyzer.

11. REAGENTS

A. Methyl cellulose, powder, USP 4000 cps - Fisher Certified Reagent #M-352 or equivalent

B. Water: deionized, particle free (<0.2 µm filtered)

C. Methyl cellulose solution: 0.002% (wt/vl) (20 ppm). Dissolve 20 ± 0.5 mg of methyl cellulose in 500 ml of deionized particle free water to make a 0.004% stock solution. Dilute 1:1 to make a working solution.

NOTE: Methyl cellulose acts as a wetting agent to aid in maintaining a uniform particle distribution as the sample dries, by greatly reducing the surface tension of water.

12. SAMPLE PREPARATION

12.1. Transfer 30 to 50 mg of talc powder to a clean 125 ml polyethylene jar.

12.2 Add 80 ml of 20 ppm methyl cellulose solution, cap and shake vigorously for one minute.

12.3. After shaking, loosen cap and ultrasonicate for 10 minutes in order to disperse the finer particles. Then shake again for one minute to produce a uniform suspension.

12.4. Immediately after shaking, uncap and remove 9.2 microliters with a micropipette.

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STANDARD TEST METHOD

SUBJECT: ANALYSIS OF POWDERED TALC FOR ASBESTIFORM MINERALS BY TRANSMISSION ELECTRON MICROSCOPY

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12.5. Transfer a 9 µl drop to a carbon film covered TEM grid. (Grid was first lightly anchored by 2 parallel strips of double-stick tape mounted 3 mm apart on a clean glass microscope slide.) Repeat to make two sample grids per talc sample.

NOTE: Do not expel the remaining 0.2 µl suspension from the micropipette tip. It tends to sputter and frequently destroys the stability of the sample drop.

12.6 Transfer slide with grids to a desiccator. (Drying time is 2-3 hours.) Do not leave the grids on the slide for more than one day as the double-stick tape may adhere too tightly.

NOTE: The talc:water ratio may need to be varied for some samples. Preparation of talc samples with a significantly finer or coarser particle size results in large differences in particle coverage on the TEM grid.

TEM ANALYSIS

13.1 Definition of fiber: An elongated particle with parallel sides and an aspect ratio $\geq$3:1. The definition employed may vary with the needs of the client.

13.2 Scan sample at 120-150X magnification to check for even dispersion of particles and to locate grid squares with optimum particle density. (Optimum particle density is particle coverage over 15-35% of the field of view.)

13.3. Scan three grid squares on each grid at 20,000X magnification and seven grid squares on each grid at 5,000X for asbestiform minerals. Each asbestiform mineral is recorded as to type (chrysotile, tremolite, anthophyllite, etc.), structure (bundle, clump, fiber) and dimensions (length x width).

13.4. Questionable fibers are examined first by SAED. The chrysotile SAED pattern is unique and diagnostic. Amphibole SAED patterns are variable but usually characteristic. Additional analysis and measurement of amphibole SAED patterns are done if warranted.

13.5. Ten percent of chrysotile fibers are checked by EDXRA for further confirmation. If the SAED pattern is not clearly diagnostic, or if it is consistent with an amphibole SAED pattern, then it is examined by EDXRA to confirm the identification or to identify the type of amphibole.

Johnson & Johnson BABY PRODUCTS COMPANY

DARD TEST METHOD

SUBJECT: ANALYSIS OF POWDERED TALC FOR ASBESTIFORM MINERALS BY TRANSMISSION ELECTRON MICROSCOPY

SUPERSEDES: ADL 1305 DATE: 3/8/89 AUTHORIZATION NO.: BCR 011362

14. CALCULATION OF RESULTS

14.1.A. Mass of chrysotile fibers: M(f)
$M(f) = \pi r^2 l \times d$
π = 3.14159
r = fiber radius
l = fiber length
d = density of chrysotile = 2.55×10^{-12} g/μm^3

14.1.B. Mass of asbestiform amphibole particles: M(a)
M(a) = l x w x th x d
l = length
w = width
th = thickness $\cong$ 0.3 width (approximation)
d = density of amphiboles = 3.3×10^{-13} g/μm^3

14.2.A. Mass of talc deposited on each TEM grid: M(s)
M(s) = T x (V/H)
T = amount of talc sampled (step 12.1)
V = volume of aliquot transferred to TEM grid (step 12.5)
H = volume of methyl cellulose solution (step 12.2)

14.2.B. Total estimated talc mass examined: M(t)
M(t) = M(s) x (N x A(s))/A(g)
N = number of grid squares examined
A(s) = area of a single TEM grid square
A(g) = area of an entire TEM grid (effective area over which a 9 microliter drop of suspension dries)

14.3. Weight percent:

$$\frac{\text{sum total of M(f) or M(a) x 100}}{M(t)}$$

15. CALCULATION OF A DETECTION LIMIT

15.1. M(dl) = A minimum quantifiable mass of asbestos fibers, based on the detection of 5 fibers (approximately 6E-13 grams, from Section 6).

15.2. Detection Limit (Weight Percent) = $\frac{M(dl) \times 100}{M(t)}$

Exhibit 13

STIMULI TO THE REVISION PROCESS

Stimuli articles do not necessarily reflect the policies of the USPC or the USP Council of Experts

Modernization of Asbestos Testing in USP Talc[a]

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ABSTRACT In response to a request from the U.S. Food and Drug Administration through the FDA Monograph Modernization Task Group, the *USP Talc* monograph is being modernized to ensure that the tests for asbestos have adequate specificity. The USP Excipients Expert Committee of the Council of Experts approved the formation of a Talc Expert Panel, which is charged with modernizing the *USP Talc* monograph. This *Stimuli* article outlines the current thinking of the USP Talc Expert Panel and discusses several test procedures and measurement criteria that are under consideration. The Talc Expert Panel is considering these procedures and criteria for recommendation to the USP Excipients Expert Committee for control of *Absence of Asbestos* in USP *Talc.* This article concludes with a summary of the adverse health effects resulting from asbestos exposure, and a proposal for updating the *Definition* and *Labeling* sections of the *USP Talc* monograph. The USP Talc Expert Panel's recommendation for revision of the test for *Absence of Asbestos* will include omission of the infrared spectroscopy test and inclusion of a revised x-ray diffraction procedure, in combination with one or more microscopic evaluations (polarized-light microscopy, transmission electron microscopy, or scanning electron microscopy).

1. INTRODUCTION

As part of USP's initiative to update and improve its monographs for drug substances and products in the *U.S. Pharmacopeia* and *National Formulary* (*USP–NF*), USP is focusing on monographs recently identified as high priority by the U.S. Food and Drug Administration (FDA) through the FDA Monograph Modernization Task Group (MMTG). On November 16, 2010, the FDA MMTG sent a letter to USP indicating the desire to modernize the high-priority *USP Talc* monograph[f] (1). The request for revision was stated as follows: *"Labeling should be revised to match the statements that are provided in the Talc FCC monograph, thereby assuring that Talc is not sourced from mines that are known to contain asbestos. Also, USP should consider revising the current tests for asbestos to ensure adequate specificity."*

The current *USP Talc* monograph contains a test for *Absence of Asbestos* that includes three procedures. Analysts are given the option to perform either *Procedure 1* or *Procedure 2,* which consist of infrared spectroscopy (*Identification Tests–General* ⟨191⟩) and x-ray diffraction (*Characterization of Crystalline and Partially Crystalline Solids by X-Ray Powder Diffraction (XRPD)* ⟨941⟩), respectively. If either test gives a positive result, then the third procedure, consisting of optical microscopy (*Optical Microscopy* ⟨776⟩) must be performed to confirm. The infrared spectroscopy (IR) and x-ray diffraction (XRD) methods, as currently written, can lead to false-negative results, which could allow talc samples with asbestos contamination to pass the *Absence of Asbestos* test in the *USP Talc* monograph. Even after applying the current USP microscopy method, the analyst cannot rule out the presence of hazardous fibers in a sample of talc. In addition, the lack of identification procedures in the optical microscopy section of the method could lead to false-positive results. This underscores the need to modernize the current monograph for two reasons: 1) both the IR and XRD methods have relatively high detection limits for asbestos, and 2) there is no known "safe" level of asbestos exposure.

In response to FDA's request to modernize the *USP Talc* monograph, the USP Excipients Expert Committee (EXC EC) formed a Talc Expert Panel (EP). The Talc EP consists of volunteer members from among talc suppliers, pharmaceutical manufacturers, regulatory and government agencies, academia, and instrument manufacturers. The charge of the EP is to update and modernize the methodology for testing that is described in the *USP Talc* monograph, thereby establishing a quality standard based upon well-defined specifications and analytical methods. This modernization will ensure that the production of talc meets an appropriate standard for the *Absence of Asbestos,* using currently available methods set below the feasible limits of detection.

This *Stimuli* article outlines the current thinking of the Talc EP and details its objectives and charge. The article then discusses several test procedures and measurement criteria under consideration by the Talc EP for recommendation to the EXC EC for the control of *Absence of Asbestos* in USP *Talc.* Section 2 discusses the derivation of talc and the formation and composition of talc deposits, whereas section 3 addresses the mineral chemistry and morphology of asbestos species potentially encountered in commercial talc deposits. Section 4 highlights the current USP test procedures for determination or analysis of asbestos in a talc matrix, while section 5 introduces methods under consideration for asbestos testing in USP *Talc.* Section 6 discusses the adverse health effects from asbestos exposure and outlines why asbestos contamination is a serious concern for USP *Talc,* thereby underscoring efforts to ensure that asbestos levels are below the feasible limit of detection when using current, state-of-the-art methodology. Finally, section 7 addresses labeling while section 8 includes the conclusions and summary.

2. TALC DERIVATION—OVERVIEW OF FORMATION AND COMPOSITION OF TALC DEPOSITS

Talc is a member of the phyllosilicate (sheet silicate) group of silicate minerals.[g] Talc's normative chemical formula is $Mg_3Si_4O_{10}(OH)_2$, with generally small amounts of substitution of other elements in more than trace amounts. These substitutions, which include Fe for Mg, Al for Si, and F for OH, generally do not have a major effect on the mineral's desirable properties. Structurally, talc is composed of a layer of Mg-O-OH in octahedral coordination sandwiched between two layers of Si-O in tetrahedral coordination. The tetrahedral-octahedral-tetrahedral units (t-o-t) are linked together by relatively weak van der Waals bonds, which result in the characteristic friability or cleavage of talc layers (*Figure 1*).

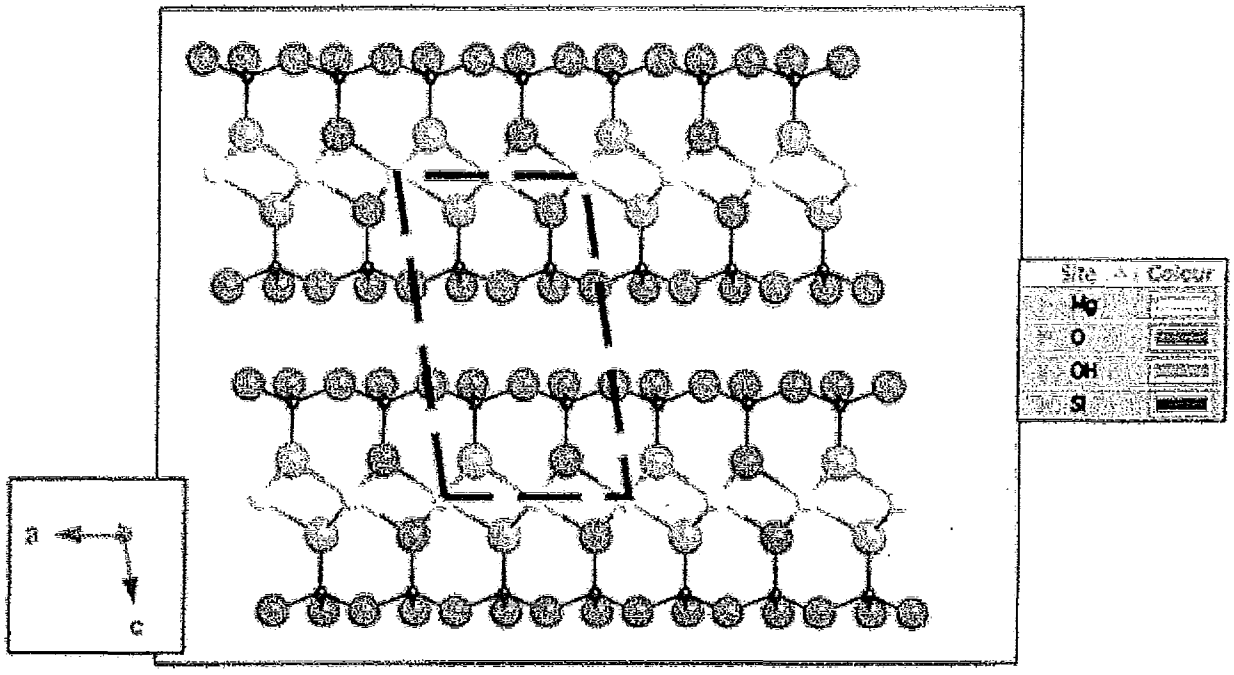
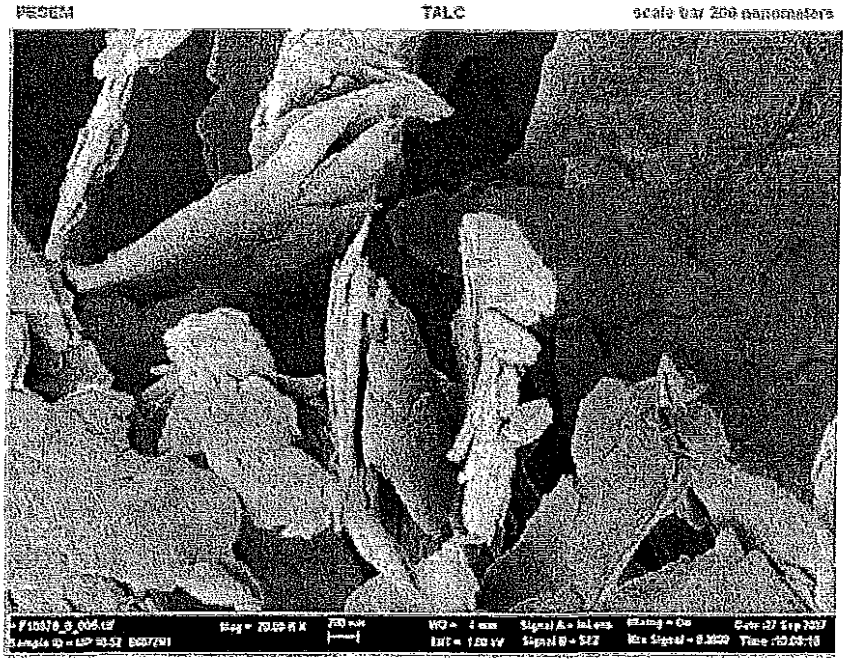


Figure 1. Crystal structure of Talc. The atoms are shown as small balls: magnesium (yellow), silicon (blue), and oxygen (red with orange for OH). Silicon, surrounded by four oxygen atoms, occupies the tetrahedral site while magnesium, surrounded by six oxygen atoms, occupies the octahedral sites of the unit cell. The unit cell (shown with the dashed black line) has dimensions of 5.3 × 9.2 × 9.5 Å. Created with CrystalMaker® version 8.7.

Talc can form when the requisite stoichiometric combination of elements is present in the initial rock (protolith) at sufficient temperature, pressure, and length of time. Talc can also form as an "up-temperature" (prograde) or "down-temperature" (retrograde) reaction product. The preservation of talc from elevated metamorphic conditions depends largely on cooling rates and the chemical flux of volatiles, especially water and carbon dioxide.

Macroscopic talc forms individual crystals and masses of crystals that separately and collectively have a "platy or plate-like" appearance (2). Talc "plates" can be relatively "small"—micrometers across—or relatively "large"—centimeters or more across (3) (*Figure 2*). Aggregates of the plates have been described as having a sample texture that is micaceous or foliated. "Foliated" means that the flattened talc grains are largely oriented as sub-parallel plates.



F18978_0_005.tif

Figure 2. Scanning electron microscopy image of typical lamellar Talc.

The physical form of talc rock is related to the geologic source (protolith) and the geologic conditions during the formation of the deposit. Talc's platelet size determines its lamellarity. Highly lamellar talc (informally classified as macrocrystalline talc) has large, stacked platelets, whereas microcrystalline talc has small, randomly oriented platelets.

The lamellar aggregates are accumulations of individual crystals that are approximately equidimensional in the equatorial plane and relatively thinner perpendicular to that plane. Occasionally, talc will grow "faster" in the shortest atomic-length direction and produce a gross shape that is elongated lamellar, which is similar to a ribbon and is informally described as "ribbon talc". When the growth in a single direction is extreme, the talc can develop a fibrous morphology.

Given the variability of pressure, temperature, and chemical flux in the geologic environment, it is not uncommon for talc to undergo alteration, via chemical and structural changes, to other minerals. Talc may even be found occasionally in a transitional state when a reaction is incomplete and frozen-in.

The four types of geologic environments most typical for talc formation are:

1. Large geographic-geologic areas (regionally) of prograde metamorphic sedimentary rocks [derived from either Mg-rich carbonates (dolomites) or shale (clay- and quartz-rich sediments)];
2. Magnesium-rich, silica-poor (ultramafic) rocks undergoing serpentinization (an alteration process that results in hydration and enrichment in silica) followed by chemical alteration arising from the influx of carbon dioxide-rich fluid;
3. Amphibole-bearing metamorphic rocks undergoing retrograde metamorphism;
4. A broad variety of protoliths undergoing local metamorphism because of elevated heating (contact metamorphic effects) (2, 4).

Talc ores are sometimes classified into two major groups based on the type of geologic environment: talc deposits with amphibole minerals as important components of the host rock, and talc deposits that are essentially "amphibole free." The majority of globally produced commercial talc is formed by the prograde sequence of sedimentary rocks (Type 1), or to a lesser extent, derivation from ultramafic igneous rocks (Type 2). *"Ultramafic is the most abundant deposit worldwide, but metasedimentary is by far the most widely exploited commercially and accounts for more than 70% of world production [of all talc, including pharmaceutical grade]"* (2).

For the remaining 25%–30%, industry experts have estimated that only a minor segment of all markets uses talc derived from amphibole-bearing metamorphic rock, and this has declined in recent years (5, 6) (*Figure 3*). Talc derived from host deposits with amphiboles is of primary concern because of the possible presence of amphibole and serpentine asbestos in the final product. Historically, tremolitic talc (Type 3) has not been used in the United States for pharmaceutical applications. *Figure 3* represents the current estimated world production of talc (5) divided into the four types.

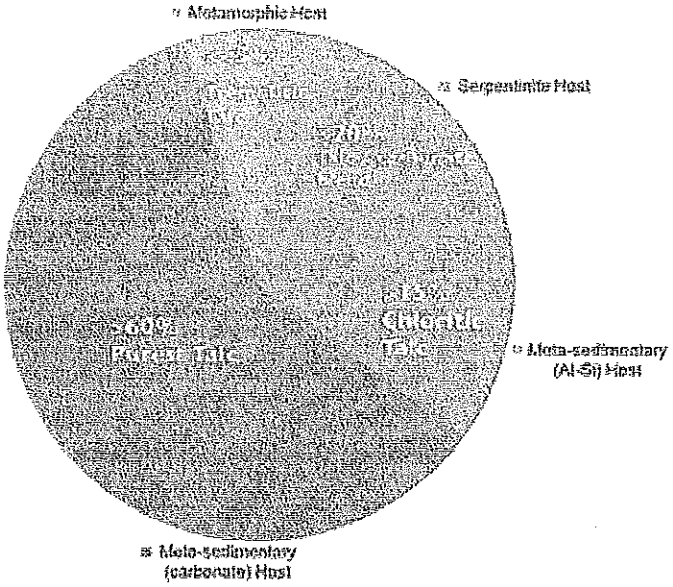


Figure 3. Current estimated world production of Talc.

3. MINERAL CHEMISTRY AND MORPHOLOGY OF ASBESTOS SPECIES POTENTIALLY ENCOUNTERED IN COMMERCIAL TALC DEPOSITS

A large number of accessory minerals may be found in talc deposits, depending on the formation conditions of the deposit. These minerals include but are not limited to dolomite, magnesite, calcite, and quartz, as well as a variety of micas, chlorites, feldspars, serpentines, and amphiboles. Of particular concern for this discussion are minerals which, under certain conditions, can occur in an asbestiform growth habit, and also the minerals that may interfere with detection of asbestos during analysis. Chlorites, typically clinochlore and chamosite, have the general composition $[(Mg,Fe)_3(Si,Al)_4O_{10}(OH)_2 \cdot (Mg,Fe)_3(OH)_6]$ and are fairly common in some talc-rich rocks and ores. Chlorite group minerals are layered silicates (phyllosilicate) that are composed of "chemical sandwiches" similar to talc, but with an additional layer of Mg-Al-O inserted into the stacking sequence. Chlorites are highly variable in composition and structural complexity, and typically do not form fibrous morphologies. Asbestos is a commercial/industrial term applied to certain naturally occurring minerals when these minerals crystallize in the asbestiform habit (generally defined as minerals with the growth form similar to commercial forms of asbestos). The commercially desirable properties of asbestos include flexibility, tensile strength, and resistance to heat, electrical conductivity, and chemical corrosion.

Certain asbestiform minerals are regulated under the rubric asbestos in numerous federal and international regulations. These regulations are based primarily on the asbestos minerals that were used commercially, and most regulations and approved analytical methods

specifically list those minerals because of early epidemiological studies linking commercial asbestos with disease. Historically, analytical methods used for identification of regulated asbestos rely on the commercial and physical properties of the minerals rather than properties that may be associated with the etiology of disease.

The asbestos minerals typically listed in regulations and methods include chrysotile, a member of the sheet-silicate group, and five amphibole minerals of the chain-silicate group. These five are "amosite" (cummingtonite-grunerite asbestos), crocidolite (riebeckite asbestos), tremolite asbestos, actinolite asbestos, and anthophyllite asbestos. Historically, chrysotile has been the most commonly used asbestos in industry (approximately 90%). Chrysotile is still being mined in a few countries; however, most countries have banned the mining of all types of asbestos because of the demonstrated and perceived health risks of the material.

Although there is general agreement in the international community, it is important to note that there is no uniformly and universally accepted "group" of asbestos minerals, nor are there universally accepted definitions for asbestos and asbestos-related particles. A tabulation of definitions for asbestos, asbestiform, and other asbestos-related terminology used in this article can be found in Lowers and Meeker (2002), and ASTM D7712-11 (7, 8).

3.1 Serpentine

Serpentine is a subgroup of minerals with the composition $[(Mg, Fe)_3(Si_2O_5(OH)_4]$. Rocks containing serpentine minerals can contain serpentine asbestos (chrysotile) if formed under specific high-shear conditions. *"There are three principal forms of serpentine—lizardite, antigorite and chrysotile—all with approximate compositions of $Mg_3Si_2O_5(OH)_4$. The most abundant is lizardite and the least is chrysotile, but the latter is perhaps the best known..." (9)*

Chrysotile is a layered silicate mineral with the nominal composition $Mg_3Si_2O_5(OH)_4$. The mineral generally forms as bundles of extremely thin fibers that can split into single units called fibrils. Chrysotile fibrils can measure as little as a few tens of nanometers in diameter, with lengths up to tens or hundreds of micrometers. These fibrils form as the mineral grows (growth habit) because of a slight atomic mismatch between alternating layers of SiO_4 tetrahedra and MgO octahedra. The atomic forces generated by this mismatch cause the layers to curve into a tight scroll during growth, thereby producing the individual fibrils.

3.2 Amphibole

The amphibole minerals have a double-chain structure composed of layers of rings of SiO_4 tetrahedra held together by alternating chains of octahedral units and interlayer cations.

Amphiboles have a general chemical formula of $A_{0-1}B_2C_5T_8O_{22}W_2$ where only the most common ions for each crystallographic site are as follows:

A = Na, K; B = Na, Ca; C = Mg, Fe, Al; T = Si, Al; W = OH, F, Cl

As suggested by the formula above, amphiboles can be extremely complex chemically, and more than 80 mineral names are currently designated, based on chemistry, by the International Mineralogical Association (IMA) (10, 11).

Amphiboles are fairly common rock-forming minerals and occur in a variety of growth habits depending on origin and conditions of formation. Single amphibole crystals are generally elongated along the *c* crystallographic direction and typically form in a prismatic (prism-like) habit. Amphiboles can also form as acicular (needle-like) crystals, and very rarely as asbestiform crystals. Amphibole asbestos fibrils can measure less than a hundred nanometers in diameter, with lengths up to tens or hundreds of micrometers. Amphibole asbestos has been mined commercially in the past, and two types, amosite and crocidolite, were widely used in a variety of commercial applications until the 1970s, when rising health concerns caused most countries to cease commercial production.

In many cases, chrysotile is easy to define and identify because of its thin fibers, unique rolled sheet structure, and simple chemistry, but the same cannot be said of amphibole asbestos. The reasons for this include the extensive chemical substitution that can occur in amphiboles, and the fact that the IMA system of nomenclature is based on mineral chemistry. Mineral identification using the IMA nomenclature requires highly accurate chemical analyses, particularly where amphibole minerals are not close to pure end-member compositions (12, 13). For example, pure end-member tremolite has the composition $Ca_2Mg_5Si_8O_{22}(OH)_2$. If, however, fluids rich in sodium, potassium, and iron were present during formation, the resulting mineral might have a composition such as $(Na,K)_{0.4}(Na,Ca)_2(Mg,Fe)_5Si_8O_{22}(OH)_2$ due to chemical substitutions. The resulting mineral, although very similar to tremolite, would be classified by the IMA as winchite. This example is significant because most current regulations list tremolite as regulated, but winchite is not even addressed, although the two minerals are associated with similar health risks (14–17).

In addition to chemistry, particle morphology is used to determine if a single amphibole particle or population of particles is asbestos. Again, the analytical methods rely on properties of commercial asbestos rather than properties directly tied to health effects. As stated above, amphiboles can form in a variety of morphologies ranging from prismatic to asbestiform.

4. CURRENT USP TEST PROCEDURES FOR DETERMINATION OR ANALYSIS OF ASBESTOS IN A TALC MATRIX

The current USP *Talc* analytical procedure for *Absence of Asbestos* utilizes either infrared spectroscopy (IR) or x-ray powder diffraction (XRD); the choice is left to the user. These initial screening methods are useful for evaluating the overall quality of the talc. Both the IR and XRD procedures, as written in the *USP Talc* monograph, are pass/fail tests that do not provide specific detection limits. If there is any indication in the test results that minerals which may have an asbestos component are present (a positive result), then the current USP method requires that the sample be examined using optical microscopy. Currently there are no standard reference materials available that can be used to document a laboratory's effectiveness in detecting asbestos in a talc matrix.

In addition, the pharmacopeial test procedures for determination or analysis of asbestos (IR, XRD, and optical microscopy) do not detect all particles thought to be hazardous, but only the subset of particles that are amenable to routine detection and quantification by the specific analytical test procedure being used. Because fibrous minerals in talc are contaminants rather than commercial materials added for their desirable properties, it is important to recognize that applying analytical methods developed for commercial asbestos may not be adequate in terms of sensitivity and specificity for determining the absence of asbestos in talc for use in pharmaceutical products (*Table 1*). In addition, other minerals (such as chlorite or kaolinite) can occur in talc; both cause interference in the detection of asbestos in talc. As with any analytical procedure, certified reference materials are necessary to properly calibrate the system.

Table 1. Current Methods for Asbestos Detection and Quantification in a Talc Matrix

Method	Description in current USP monograph	Advantages	Disadvantages
IR absorption spectroscopy	758 ± 1 cm^{-1}, may indicate the presence of tremolite or chlorite. If the absorption band remains after ignition of the substance at 850° for at least 30 min, this indicates the presence of tremolite. In the range 600 cm^{-1} to 650 cm^{-1} using scale expansion, any absorption band or shoulder may indicate the presence of serpentines.	Instrumentation is typically available for companies that need to perform pharmaceutical testing.	Cannot distinguish asbestos from non-asbestos forms of the same mineral. The method is subject to interferences with other minerals. Detection limit is unknown.
X-ray diffraction	The presence of amphiboles is detected by a diffraction peak at 10.5 ± 0.1° 2Θ, and the presence of serpentines is detected by diffraction peaks at	Important in fully characterizing mineral assemblage. Provides information about bulk purity.	Cannot distinguish asbestos from non-asbestos forms of the same mineral. Limit of detection

	24.3 ± 0.1° 2Θ to 12.1 ± 0.1° 2Θ.	Can give information about the origin of the talc deposit and the associated risk. Can indicate if problematic levels of any phase are present.	may be too high for public health and regulatory purposes. Detection limit of serpentine is severely affected by presence of chlorite. May give false-negative result if used as a screening method.
Optical microscopy	The presence of suspect fibers is inferred from the occurrence of particles with length-to-width ratios in the range from 20:1 to 100:1, or higher for fibers longer than 5 µm.	Identification considers particle morphology.	Particles of milled material may be disaggregated and inconsistent with typical asbestos morphology. Particles of milled material may be below resolution limit. Due to lack of identification procedures, may give a false-positive result. Limit of detection may be too high for public health and regulatory purposes.

5. METHODS UNDER CONSIDERATION FOR ASBESTOS TESTING IN TALC

Talc analytical methods have been a subject of development by ASTM International (18). The Asbestos Analytical Committee (D22.07) has been working on a series of detailed procedures covering XRD, polarized-light microscopy (PLM), and transmission electron microscopy (TEM) analyses, specifically for pharmaceutical Talc. To date, drafts of all three procedures have been reviewed by the ASTM committee, although the TEM method has progressed the furthest. The Expert Panel is monitoring these methods and is working with ASTM, where appropriate, to further their development.

5.1 X-ray Diffraction

XRD is used for qualitative determination (identification) and quantitative determination (weight percent) of crystalline substances. The three-dimensional structure of crystalline substances generates elastic x-ray scattering called diffraction, and satisfies the Bragg Equation:

$$n\lambda = 2d\sin\theta$$

where n is an integer called the order of the reflection; lambda (λ) is the wavelength of the characteristic line of the tube anode material, typically Cu Kα; d is the interplanar spacing of given crystal planes of a crystal; and theta (θ) is the x-ray incidence angle (Bragg angle) under a given instrument geometry. The Bragg equation represents an inverse relationship where low theta (θ) values would have a corresponding high d-spacing (usually expressed in Angstroms) and vice versa. When using XRD, consideration should be given to the differences in the particle size distribution, crystallinity, and interferences, among others. Matrix-matching of the standard and test materials and their preparations are important criteria to meet in order to achieve precise and accurate results. XRD provides an important initial screening of the talc product for ancillary mineral phases, especially for those of total amphibole and total serpentine. Amphibole and serpentine minerals are typically non-asbestiform, but they can exist more rarely as an asbestiform variety. However, XRD does not delineate the non-asbestiform and asbestiform varieties of amphibole or serpentine; therefore, XRD should be combined with one or more microscopic techniques. For total amphibole, conventional XRD provides a qualitative non-detect at < 0.5% in talc. XRD performed with extended count times can achieve lower detection limits such as < 0.1%. For serpentine, XRD provides qualitative and quantitative detection limits that will vary because of interference from the chlorite group minerals; here, detection limits could be as low as 0.1% or as high as 2%.

5.2 Polarized Light Microscopy

Polarized-light microscopy (PLM) is used to identify a substance based on its optical properties. The fibers in talc product that satisfy pre-defined criteria for optical properties including refractive index, sign of elongation, and extinction angle, as well as dimensions and morphology, will be identified as asbestos based on specific regulatory methods. PLM can be used for quantitation of asbestos, often using a "point-count" method (19). The detection limit can be improved by increasing the number of points counted. Accurate PLM quantitation depends on resolution and identification of asbestos and non-asbestos particles. The fibers with particle sizes below the wavelength of illumination cannot be resolved by PLM. The unresolved fibers are not counted, which may lead to false-negative results. For this reason,

amphibole and serpentine detected by XRD may be unresolved by PLM.

5.3 Electron Microscopy

Electron microscopy, including transmission electron microscopy (TEM) and scanning electron microscopy (SEM), overcomes the resolution limitations of PLM and has the ability to detect extremely small asbestos fibers. The minimum fiber width that can be routinely characterized by TEM is on the order of 0.03 µm (19, 20), corresponding to the typical width of single chrysotile fibrils. TEM is the only method that can accomplish this, although the modern field emission SEM can approach this capability. TEM and SEM provide elemental composition data through energy dispersive x-ray spectroscopy (EDS), an important component of the identification of the mineral. TEM also provides information on crystalline structure through selected area electron diffraction (SAED), and recent developments using electron back-scattered diffraction (EBSD) may enable analysts to derive similar crystallographic information with SEM (21). In a recent review of the draft National Institute for Occupational Safety and Health (NIOSH) roadmap for asbestos research, the Institute of Medicine of the National Academies stated: *"The need to develop new [analytical] methods based on electron microbeam techniques is critical and should not be limited by existing regulatory constraints or existing policy."* (14, 15) A comparison of the methods described above, outlining their advantages and disadvantages, is presented in *Table 2*.

Table 2. New Microscopy Methods Under Consideration

Method	Description	Advantages	Disadvantages
Polarizing light microscopy	The presence of asbestos is confirmed by the occurrence of particles with asbestos morphology and their identification as an asbestos mineral based on optical properties/dispersion staining.	Identification is based on morphology and phase determination, which can be conclusive. Particles characterized by PLM are in the size range where they are easily distinguished as asbestos, compared with non-asbestos. Good method for larger-size products	Normal quantitation limit may be too high for public health and regulatory purposes, if concentration techniques are not used. Particles of milled material (< 5 µm) may be below resolution limit.

		typical of personal care talc products.	
Scanning electron microscopy (SEM)	The presence of asbestos is confirmed by the occurrence of particles with asbestos morphology that are identified as an asbestos mineral by EDS elemental analysis.	A larger sample size (µg range) is analyzed, relative to TEM. Identification is based on morphology and elemental analysis. Resolution is better than with PLM. Capable of disclosing surface morphology.	Fibrils of chrysotile may be below the resolution limit of older microscopes. Because it is a presumed identification based on chemistry and morphology alone, the test may give a false-positive result. Structural information methods are currently in development. Interferences include talc/anthophyllite, etc.
Transmission electron microscopy (TEM)	The presence of asbestos is confirmed by the occurrence of particles with asbestos morphology that are identified as an asbestos mineral by EDS elemental analysis and electron diffraction.	Identification is based on morphology, elemental analysis, and electron diffraction (structural information). May be the only method with resolution high enough to routinely detect fibrils of chrysotile.	May be prohibitive for quality control due to protracted prep/analysis time, high cost, irreproducibility, and small sample size (ng range). May miss the larger fibers associated with amphibole asbestos (false negative).

5.4 Additional Sample Preparation/Concentration Techniques

Detection of asbestos in talc by the instrumental methods outlined above can be enhanced through the concentration of asbestos particles or separation of asbestos from obscuring or confounding particles. Several sample preparation techniques are being evaluated; each targets a specific type of particle to analyze. These techniques are: 1) air elutriation, for the purpose of evaluating the fraction of particles that may become airborne; 2) aqueous elutriation, also for evaluating particles that may become airborne; and 3) wet sieving, which effectively concentrates asbestos in the larger, more easily characterized size fraction and lowers the overall detection limit of the methods.

5.4.1 FLUIDIZED BED ASBESTOS SEGREGATOR

The fluidized bed asbestos segregator (FBAS) is a sample preparation instrument that utilizes air elutriation to separate particles on the basis of aerodynamic diameter, which correlates positively with particle size and inversely with particle density. Asbestos structures (fibers, fiber bundles, and fibers/bundles in matrices) are collected on a filter which can then be analyzed by TEM or other appropriate microscopic techniques. The performance of the FBAS preparation method was recently evaluated by the U.S. EPA using a variety of performance-evaluation (PE) standards that spanned different matrix materials (soil and vermiculite) and different types of asbestos (chrysotile and amphibole). Results for these PE standards show that there is an approximately linear relationship between the concentration of asbestos in the PE standard (as mass percent) and the mean concentration estimated by the TEM analysis following preparation by FBAS, expressed as asbestos structures captured on the filter per gram of test material (s/g). Method detection limits achieved in these studies ranged from 0.002% to 0.005% by weight, which is approximately 100 times lower than the detection limits that are usually achieved using other analytical methods for asbestos in soil and other solid media.

The FBAS unit is compact, fitting into a standard laboratory fume hood, and components of the unit are relatively easy to decontaminate or are disposable. The FBAS unit construction and operation costs are relatively low, and sample throughput is high (up to 20 samples per day). Current research using the FBAS unit is ongoing, and an interlaboratory validation study is in progress (15). Although the FBAS method has not yet been applied to the evaluation of asbestos contamination in a talc matrix, this approach appears to have promise as a fairly inexpensive and highly sensitive method for the identification of low levels of asbestos in talc (22).

5.4.2 AQUEOUS ELUTRIATION

This elutriation technique uses water rather than air to separate particles (23, 24). A sample is suspended in a funnel of water which is constantly flushed with water coming in from the bottom. The flow rate is controlled to flush out of the top of the funnel only particles smaller than a pre-determined aerodynamic diameter. This portion is filtered and prepared for TEM analysis. The use of water removes any undesirable electrostatic interactions that can occur in air samples. Method detection limits vary based on the duration of elutriation and the differences in the aerodynamic diameters of the target particles and matrix particles, as is the case for FBAS.

5.4.3 WET SIEVING TECHNIQUE

The technique of wet sieving a milled talc product capitalizes on the natural characteristics of asbestos (i.e., flexibility and durability, which make it resistant to grinding). After milling, the sieve acts to concentrate any asbestos present by removing the easier-to-grind matrix material (i.e., talc with a softness of 1 on the Mohs Scale of Hardness). Although the size fraction analyzed is not that which includes the finest particles, this technique is an easy and cost-effective way to indicate whether or not asbestos is present. Studies have shown that even in the finest micronized talc (median particle size of 1 µm) asbestos was easily detected by conventional microscopy techniques. The effect of concentration also lowers the detection limit, for example samples with 100–500 ppm asbestos—confirmed by TEM—were effectively detected by PLM (25). In addition, asbestos particles in the larger-size fraction are more likely to maintain the unique characteristics of asbestos, which facilitates an unambiguous identification. An inexpensive, standard 325- to 400-mesh laboratory sieve is used with standard laboratory procedures to achieve these results.

6. ADVERSE HEALTH EFFECTS FROM ASBESTOS EXPOSURE

Health effects associated with workplace asbestos fiber exposures were clearly identified in the early part of the twentieth century and continue to be further elucidated through research and ongoing health studies. The major non-cancer health effects associated with airborne asbestos exposure increase with increasing levels of exposure and include pleural effusions, pleural fibrosis [both circumscribed disease (plaques) and diffuse disease], and interstitial fibrosis (also known as "asbestosis"). The observable onset of these conditions, which can occur in combination, usually takes more than 20 years from initial exposure (latency period) and can progress in severity from asymptomatic to disabling and fatal, despite cessation of exposure years earlier (26).

The risk for asbestos-related malignancies also rises with increasing levels of exposure. Among these malignancies, lung cancer is the most common. However, the types of lung cancer observed with asbestos exposure are similar to those seen with cigarette smoking, and often may not be identified as asbestos-related given the high prevalence of smoking exposures. It should be noted that the risk for lung cancer is greatly increased by the combination of asbestos and smoking exposures. Mesothelioma is a very rare cancer of the pleura (outer lining) of the lungs and abdomen (peritoneum) that is predominantly caused by asbestos exposure; it is not related to smoking and usually occurs 20–40 years after the initial exposure. According to the Centers for Disease Control and Prevention, the annual U.S. death rate due to mesothelioma is about 14 per million people for those over 25 years of age (27). The risk for mesothelioma increases with greater asbestos exposure, however, there are numerous cases of seemingly inconsequential, low-dose paraoccupational and

environmental asbestos exposures that are associated with this malignancy. Per the International Agency for Research on Cancers (IARC), there is sufficient evidence in humans that all forms of asbestos (chrysotile, crocidolite, amosite, tremolite, actinolite, and anthophyllite) cause mesothelioma and cancer of the lung, larynx, and ovary. Positive associations also have been observed between exposure to all forms of asbestos and cancer of the pharynx, stomach, and colorectum (19, 28).

Although the relationship between airborne asbestos exposure and respiratory disease is clear, associations between ingestion of asbestos fibers and gastrointestinal (GI) cancers, or other cancers due to translocation of fibers from the pulmonary or gastrointestinal tract, is more difficult to assess. Studies in humans and animal models have provided differing evidence for ingestion-related GI cancers, which were estimated to be elevated by the EPA and the National Academy of Sciences (29).

There are currently no established safe levels of asbestos exposure. This underscores the efforts of the Talc EP to identify strategies and methods for reducing the potential for asbestos contamination of talc to the lowest feasible levels. More effective analytical approaches are needed to achieve much lower levels of detection than those traditionally used to evaluate asbestos contamination of bulk materials. The existing methods are not necessarily adequate for assessing the potential health risks of these materials. Research by the U.S. EPA and others has shown that disturbance of matrices (e.g., soil, vermiculite insulation) containing asbestos concentrations identified by the lower detection limits of PLM—well below 1% asbestos by weight, the limit historically used by the U.S. EPA to define an Asbestos Containing Material)—can generate potentially hazardous exposures (30–32). This issue, while not currently evaluated, may be particularly relevant for the talc used in powders and cosmetics.

Current standards and recommendations have generally focused on controlling asbestos mineral fiber exposures (chrysotile, crocidolite, amosite, anthophyllite asbestos, tremolite asbestos, and actinolite asbestos) by using optical microscopy methods and counting all fibers with specified aspect ratios (e.g., 3:1 or greater) and fiber lengths (e.g., > 5 µm). However, the specified dimensional criteria (length and aspect ratio) used for the quantification of asbestos may not be optimal for protecting exposed individuals, as these criteria are not based solely on health concerns (15). Animal studies and epidemiologic studies have found that various forms of asbestos, or certain dimensional characteristics of fiber exposures, were associated with different responses of the respiratory tract and different potency for disease such as mesothelioma (15, 28). Generally, the accepted physiochemical properties of asbestos fibers that are related to pathogenicity include 1) fiber dimensions (i.e., length, width, aspect ratio), 2) surface chemistry, 3) surface area, and 4) biopersistence. Although the latter three properties are not reflected in the current analytical methods for

identification of asbestos (15, 28, 33), efforts are underway to better understand the inter-relationships of these physiochemical properties in association with observed health effects. For example, researchers from the U.S. EPA and other federal agencies have recently shown that the role of surface area, as well as other factors, is important in understanding the toxicity of asbestos and other hazardous elongate mineral particles (33). Also, exposures to certain nonregulated minerals such as fibrous forms of winchite, richterite, and antigorite are of concern. Recent studies have found that such exposures are associated with increased risks of mesothelioma and other asbestos-related diseases (15, 16, 34, 35).

The USP Talc Expert Panel agrees that exposure risks can and should be mitigated by revising USP methods, which will then allow for much lower detection limits for asbestos, and if warranted, other mineral fibers. The Panel is not proposing to identify and exclude all mineral fibers under this standard, but these methods appear capable of identifying other fibers that appear to be hazardous.

7. LABELING

FDA's November 2010 letter included the following requests: *"Labeling should be revised to match the statements that are provided in the Talc FCC monograph, thereby assuring that Talc is not sourced from mines that are known to contain asbestos. Also, USP should consider revising the current tests for asbestos to ensure adequate specificity."*

However, the existing FCC description (36) is informational, qualitative, and not easily defined. Further, the FCC monograph does not include a labeling statement or any methodology for asbestos detection.

It is the conclusion of the Talc Expert Panel that mine suitability as a source of talc is not subject to USP quality standards. Rather, it is the responsibility of the talc supplier to supply a product that is asbestos free and can meet the USP compendial standards.

Based on the above, the panel recommends updating statements in the definition and/or labeling sections to indicate that talc containing (detectable) asbestos is not pharmaceutical grade.

8. CONCLUSIONS AND SUMMARY

Proposed updates to the current official harmonized *USP Talc* monograph's test for the *Absence of Asbestos* will incorporate current analysis protocol:

- Pass-fail must include microscopy follow-up to XRD.
- Definitive microscopic identification and characterization of asbestos/mineral fibers is critical in the determination of the presence/absence of asbestos.

XRD or IR analysis provides for the detection of total amphibole or total serpentine. Failure to detect amphibole or serpentine by XRD or IR does not provide adequate assurance regarding the absence of asbestos contamination.

The USP Talc Expert Panel's recommendation for revision of the test for *Absence of Asbestos* will include omission of the IR spectroscopy test and inclusion of a revised XRD procedure in combination with one or more microscopic evaluations (PLM, TEM, or SEM). The panel also recommends including additional sample preparation/concentration methods to improve the feasible limits of detection as indicated (see section 5.4).

These recommendations for method revision and labeling will help to ensure that talc does not contain asbestos or other hazardous mineral fiber contamination such as winchite or richterite as determined by current state-of-the-art procedures. The analytical approach recommended by this Expert Panel, consistent with the industry norm at present, should continue to ensure that current supplies of talc are of the highest quality, in accordance with current best practice procedures.

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[a] Disclaimer: The views expressed in this stimuli article are those of the authors and do not reflect the official views and policies of the USPC, USP Council of Experts, or the authors' institutions including FDA.

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[f] In accordance with *USP General Notices and Requirements, section 2.20, Official Articles,* the USP *Talc* article is capitalized.

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[g] Geologists define a mineral as a naturally occurring, homogeneous solid, inorganically formed, with a definite chemical composition and an ordered and periodic atomic arrangement.

Exhibit 14

[CANCER RESEARCH 55, 2232–2235, June 1, 1995]

Advances in Brief

Crocidolite Asbestos Fibers in Smoke from Original Kent Cigarettes[1]

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Abstract

The original version of the Kent Micronite cigarette filter used crocidolite, a form of asbestos, from 1952 until at least mid-1956. Cigarettes from intact, unopened packs of the brand from this period were examined. One filter contained approximately 10 mg of crocidolite. Crocidolite structures were found in the mainstream smoke from the first two puffs of each cigarette smoked. At the observed rates of asbestos release, a person smoking a pack of these cigarettes each day would take in more than 131 million crocidolite structures longer than 5 μm in 1 year. These observations suggest that people who smoked the original version of this cigarette should be warned of their possible substantial exposure to crocidolite during the 1950s.

Introduction

The initial version of the filter in Kent cigarettes used crocidolite asbestos as the filtering agent (1). The filter consisted of rolled crepe paper interleaved with a loose mass of large diameter organic fibers that had been mixed mechanically with small diameter crocidolite fibers (Fig. 1; Refs. 2–5). There was no barrier or secondary filter between the end of this filter and the customer's mouth. This design was used from the introduction of the brand into test markets in March 1952 through at least May 1956 (6). In all, an estimated 11.7 billion cigarettes (585 million packs) were sold in the United States using this design (7) with advertising that emphasized the "health protection" supposedly provided by the filter (8).

The availability of unopened packs of original Kent cigarettes from cigarette pack collectors has permitted us to confirm the presence of crocidolite in the filters and to determine whether asbestos fibers entered the mainstream smoke from these cigarettes.

Materials and Methods

Cigarettes. Cigarettes from an unopened pack of Kent cigarettes with intact cellophane bearing a Pennsylvania tax stamp, dated by its federal tax stamp as having been made in 1955 or later, were used to confirm the presence of crocidolite, to measure the amount of asbestos in a single filter, to conduct a preliminary smoking experiment, and to examine the proximal filter end by scanning electron microscopy. The filters appeared undisturbed and in good condition. Cigarettes from an unopened pack of Kent cigarettes with intact cellophane bearing a Vermont state tax stamp, dated by its federal tax stamp as having been made in 1952, were used in the smoking experiments. These cigarettes were in excellent condition. There was no mold or discoloration, and the filters appeared intact and undisturbed.

Asbestos Content of the Filter by Weight. A filter was removed with a scalpel, weighed to 0.1 mg, and ashed in a muffle furnace at 450–500°C overnight. The weighed residue is reported as the mass of asbestos in the filter.

Preliminary Smoking Experiment. Four cigarettes were smoked on a standard, piston-type smoking machine at the American Health Foundation (Borgwaldt smoking machine RM 1/G, Hamburg, Germany; Ref. 9). The standard protocol was modified by the use of MCE[3] filters instead of Cambridge filters to trap smoke particles. Grids prepared for TEM from the MCE filters were contaminated heavily with glass fibers identical to those that comprise Cambridge filters. This contamination made it impossible to analyze these grids for crocidolite.

Smoking Apparatus (Smoker). Because of the contamination problem we encountered using the conventional smoking machine, a piston-type smoker was designed to smoke the cigarettes and collect smoke particles. The smoker consisted of a modified, new, 30-ml syringe (Becton Dickinson, Norcross, GA). The receiving end was bored out to 9 mm, and the intact syringe was washed out with xylene to remove the Dow 360 medical silicone lubricant. It was then relubricated with glycerol.

Treatments and Smoking. Cigarettes were humidified to a moisture content of 9 ± 1% (SD; Ref. 10). Two puffs were taken from each of nine cigarettes. Before insertion into the smoker, 3 of the cigarette filters were rolled (360° between the thumb and forefinger with 1–2 mm inward deflection) and 3 were pinched (once between thumb and forefinger with 1 mm inward deflection). The remaining three were not manipulated prior to insertion into the apparatus. Smoking was accomplished by inserting a cigarette into the receiving end of a syringe, sealing the cigarette at the syringe with commercially available silicone sealant, suspending the smoker assembly vertically, and lighting the cigarette with a butane lighter. After lighting, the plunger was pulled to 30 ± 1 ml within 1–2 seconds, and the cigarette was extinguished by capping with a preformed aluminum foil snuffer. The entire assembly was allowed to stand vertically for 90 min. For second puff experiments, the cigarette was transferred to a second syringe, sealed, relit, and smoked as already described. After standing, the cigarette and plunger were removed carefully.

Puff residue inside the smoker was prepared and examined as follows: the plunger was reinserted; the syringe assembly was filled with 20 ml of deionized distilled H_2O; capped with parafilm; hand shaken; and allowed to stand for 30 min. After standing, syringes were hand shaken, filled to 30 ml with deionized distilled H_2O, and the contents were pulled through a 13-mm 0.22-μm pore-size MCE filter. The MCE filter was then prepared for TEM analysis according to our laboratory's modification of the standard EPA protocol (11). Six control samples, 1991 Kent filter cigarettes, were smoked and analyzed in the same manner as 1950s cigarettes. Three blank samples consisting of laboratory air drawn through the smoking device, as well as six concurrent laboratory blanks, were also analyzed.

Microscopy of Filters and Smoke. To determine filter fiber types, fiber samples from the filter of a cigarette end were placed on a glass slide in refractive indices immersion liquid Series B 1.680 (Cargille Laboratories, Cedar Grove, NJ) and examined by polarizing light microscopy for morphology extinction, pleochroism, and sign of elongation.

To identify fiber types and fiber arrangement at the mouthpiece end of the cigarette filter, filters were removed from smoked and unsmoked cigarettes and examined with a Hitachi S-800 field emission scanning electron microscope. Fiber chemistry was determined with the use of a Tracor Northern TN 5500 EDXA system.

Puff residue was examined for asbestos structures content with the use of either a JEOL 1200 EX or Hitachi 7110 TEM at a magnification of ×20,000. Asbestos structures were identified positively by their morphology, by their chemistry with the use of a Tracor Northern TN 5500 or Kevex Delta Class 5

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[3] The abbreviations used are: MCE, mixed cellulose ester; TEM, transmission electron microscopy; EDXA, energy-dispersive X-ray analysis system.

Fig. 1. Mouthpiece end of an original Kent Micronite filter.

energy dispersive X-ray analysis system, and by their crystalline structure with the use of selected area electron diffraction. Asbestos structures were counted and classified according to standard EPA protocols (11).

Results

Fig. 1 shows the mouthpiece end of an original Kent cigarette Micronite filter. Fibers comprising the web between crepe paper layers were of two types, organic and inorganic. The inorganic fibers were confirmed by polarizing light microscopy to be crocidolite asbestos; a single filter contained 10 mg of crocidolite. On the basis of a fiber length of 5 μm, a diameter of 0.1 μm, and a density of 3.2 gm/cm^3, 1 filter could contain as much as 80 billion crocidolite asbestos fibers.

Under scanning electron microscopy, the organic fibers had the appearance of typical cellulose acetate. The dense fibers and fiber aggregates protruding from the mouthpiece of the filter seen with the use of scanning electron microscopy were confirmed to be crocidolite asbestos by EDXA (Figs. 2 and 3).

TEM analysis of puff residue showed that all cigarettes smoked released asbestos structures as fibers or fiber aggregates (clusters, bundles, or matrices of fibers) in both puffs (Fig. 4). These structures were identified positively as crocidolite by EDXA and selected area electron diffraction.

Data from the first and second puff experiments are shown in Table 1. Rolled cigarettes released from 64,410 to 156,600 total crocidolite structures in 2 puffs, and between 12,960 and 17,070 of these were 5 μm or longer. Pinched cigarettes released from 28,110 to 132,060 total crocidolite structures in 2 puffs, and between 4,950 and 6,330 of these were 5 μm or longer. Nonmanipulated cigarettes released from 76,200 to 728,520 total crocidolite structures in 2 puffs, and between 3,690 and 35,250 of these were 5 μm or longer. Overall, a mean of 170,240 crocidolite structures, including 18,020 structures 5 μm or more in length, were released in two puffs from a single cigarette. No

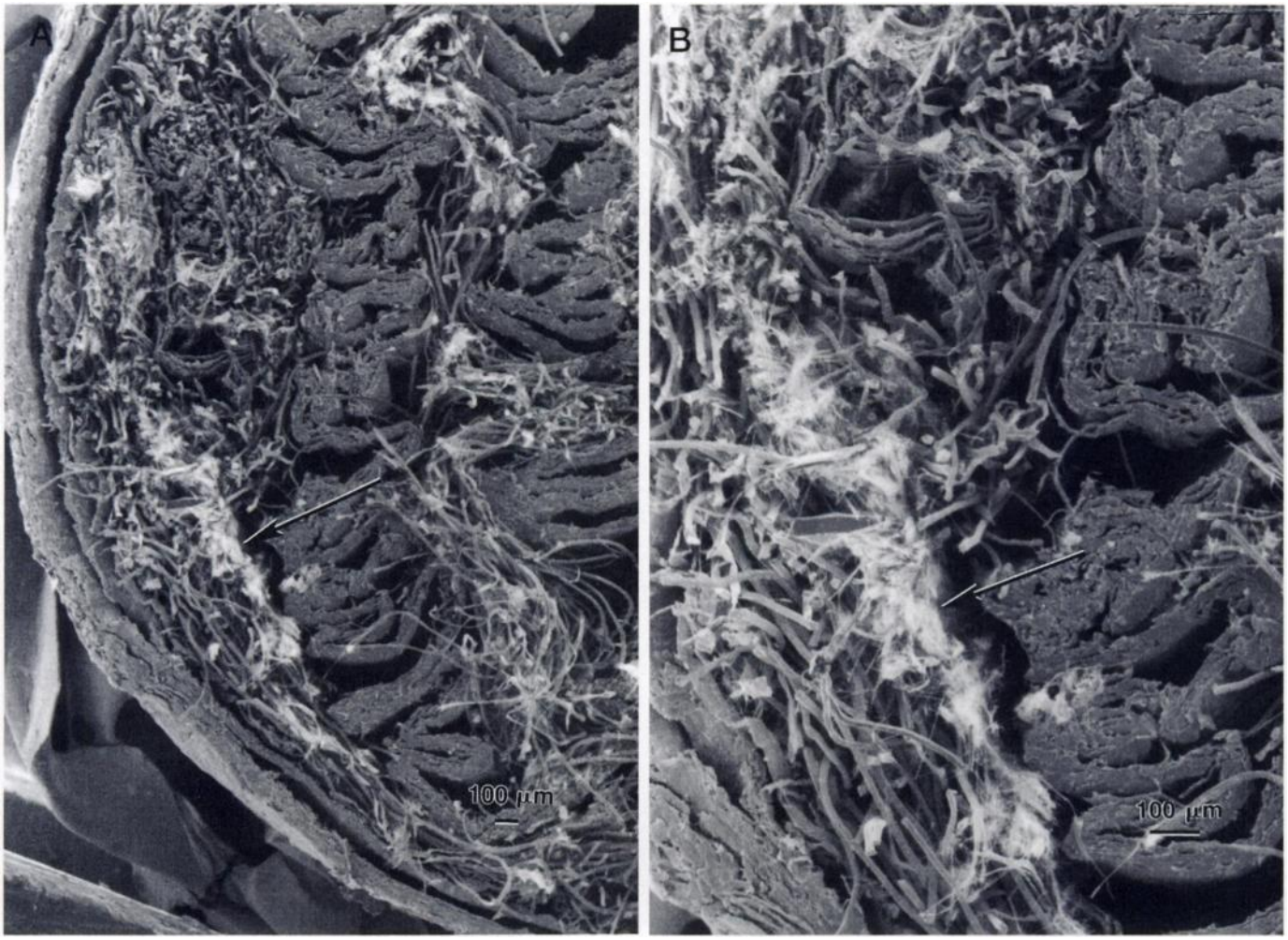

Fig. 2. Face view of mouthpiece end of filter. *Arrows*, crocidolite asbestos. *A* and *B*, scanning electron microscopy.

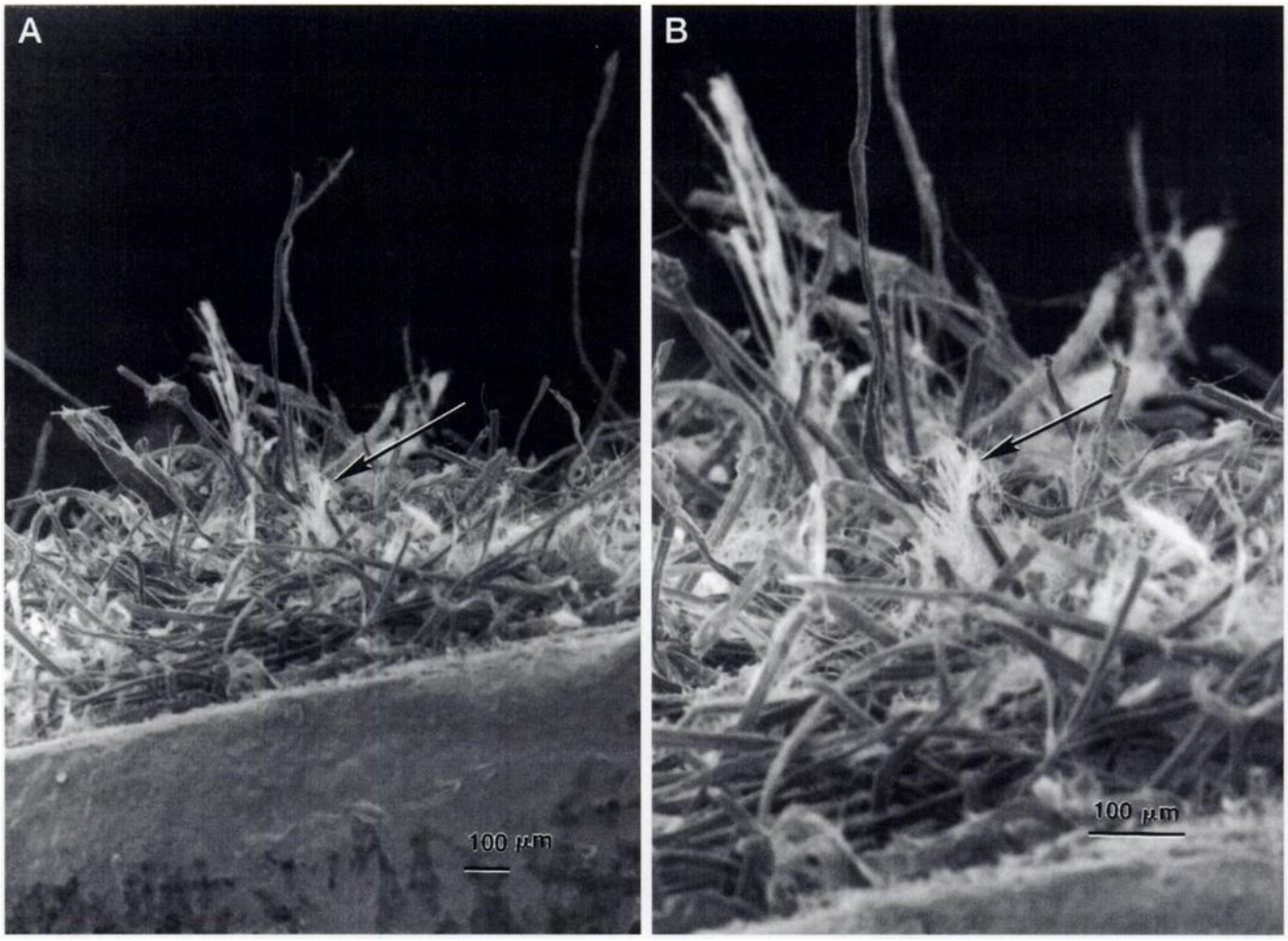

Fig. 3. Side view of mouthpiece end of filter. *Arrows*, crocidolite asbestos. *A* and *B*, scanning electron microscopy.

crocidolite structures were observed in any of the control or blank samples.

Discussion

Our data confirm the results of two series of TEM-based tests of Kent cigarette smoke performed in early 1954, one TEM series performed by Althea Revere (Life Extension Foundation), the other by Douglas Halgren and Dr. Ernest Fullam (Ernest Fullam Laboratories, Schenectady, New York). While both original reports have been lost, it is clear from other documentation that both laboratories observed asbestos structures in mainstream smoke from Kent cigarettes (12). These two studies were among the first to use electron microscopy to detect individual asbestos fibers. The present work extends these earlier studies by quantifying the amount of crocidolite released from the filter during the smoking process.

The number of structures observed in mainstream smoke in the present study is substantial. Extrapolating the observed average structure count to smoking 1 pack/day for 1 year, there would be 1.24 billion structures in the first 2 puffs of 7,300 cigarettes, and 132 million of these structures would be longer than 5 μm. Some authors have suggested that asbestos structures longer than 5 μm have a greater carcinogenic potential than do structures less than 5 μm (13).

Although a large number of fibers entered the smoke stream, only a small fraction of the total amount of crocidolite in the filter was released. We estimate that the average number of structures observed in the first two puffs represents less than 0.001% of the crocidolite in a single original Micronite filter.

While asbestos was found in smoke from each cigarette, there was substantial cigarette to cigarette variability in the amount released. Rolling or pinching the filters prior to smoking did not seem to influence the results. The observed variability may be a consequence of the design and manufacturing process of the filter itself. Crocidolite was mixed mechanically with textile fibers, and this mixture was then

Table 1 *Asbestos structures per puff (30 ml)*

	PUFF 1		PUFF 2		BOTH PUFFS	
Cigarette	Total structures	Structures >5 μm	Total structures	Structures >5 μm	Total structures	Structures >5 μm
Rolled, smoked						
1	37,860	2,280	118,740	10,680	156,600	12,960
2	62,430	13,740	1,980	1,320	64,410	15,060
3	89,700	16,140	23,250	930	112,950	17,070
Pinched, smoked						
4	27,570	6,330	540	0	28,110	6,330
5	114,600	17,190	17,460	1,740	132,060	18,930
6	14,130	2,250	29,970	2,700	44,100	4,950
Nonmanipulated smoked						
7	112,860	16,920	76,350	18,330	189,210	35,250
8	459,840	18,390	268,680	29,550	728,520	47,940
9	11,760	480	64,440	3,210	76,200	3,690
Mean	103,417	10,413	66,823	7,607	170,240	18,020

Fig. 4. Crocidolite asbestos in mainstream smoke from an original Kent cigarette. TEM; ×3000.

layered onto a paper backing. Several such layers of fibers and paper were then twisted and rolled into a filter (2–5). This mechanical process would have resulted in crocidolite fibers in each filter being distributed unevenly at the proximal end. In some cases, the disposition of fibers would have favored a large release, while in others, the geometry would have permitted less to enter the mainstream smoke. Our scanning electron microscopy observations confirm the plausibility of this explanation (Figs. 2 and 3).

Our data probably underestimate the amount of crocidolite released in an actual smoking situation for 3 reasons: (*a*) these tests examined only smoke from the first 2 puffs, and there was still substantial release of asbestos during the second puff; (*b*) the numbers given, in conformance with EPA counting rules (11), reflect "structures" and not "fibers." Overall, 18.7% of the structures observed were aggregates rather than individual fibers. An aggregate includes at least 3 and often hundreds of fibers; and (*c*) the structures recovered from the smoking apparatus are only those that had settled on the interior of the syringe and had become suspended in the wash water. Structures that remained adherent to the wall were not counted.

Of all the forms of asbestos, crocidolite is implicated most strongly as causing mesothelioma (14–16), and the risk of mesothelioma in exposed populations reaches its peak 35 to 40 years after exposure (13). An epidemic of asbestosis, lung cancer, and mesothelioma has occurred among workers at the factory where the filters for the original Kent cigarette were made (17).

Recently, Pauly *et al.* (18) have shown that 12 popular brands of cigarettes shed filter material into the smoke stream and that those fibers are deposited into the lungs during the smoking experience. On the basis of results from the present study and the study of Pauly *et al.* (18), in conjunction with the earlier work by Revere, Fullam, and Halgren (12), we conclude that the original Kent cigarettes tested at our laboratory accurately represent how the cigarettes would have released crocidolite fibers if tested in the same manner in the early 1950s. This in turn strongly suggests that there is an increased risk of mesothelioma among people who smoked these cigarettes during that time point.

While the original version of Kent was on the market, the brand had an overall market share of 0.72% (7). Its best year was 1954, when it accounted for 1.1% of the market. In that year, about 550,000 packs were sold each day. Thus, up to several hundred thousand people still alive were exposed to substantial amounts of crocidolite from smoking this cigarette.

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Exhibit 15

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Fiber Release During the Removal of Asbestos-Containing Gaskets: A Work Practice Simulation

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Work practice studies were conducted involving the removal of asbestos-containing sheet gaskets from steam flanges. These studies were performed to determine potential exposure levels to individuals who have worked with these types of materials in the past and may still work with these products today. The work practices were conducted inside an exposure characterization laboratory (ECL) and were performed by scraping and wire brushing, chrysotile-containing (65% to 85%) sheet gaskets from a number of used steam flanges. Airborne asbestos levels were measured by phase contrast microscopy (PCM) and transmission electron microscopy (TEM) for the personnel and area air samples collected during the study. These workplace simulations showed substantial asbestos fiber release using scraping, hand wire brushing, and power wire brushing techniques during the gasket removal process. The range of concentration was 2.1 to 31.0 fibers/cc greater than 5 micrometers when measured by PCM. These results contrasted with the few reported results in the published literature where lower airborne asbestos levels were reported. In these studies the airborne asbestos fiber levels measured in many of the samples exceeded all current and historical Occupational Safety and Health Administration (OSHA) excursion limits (15–30 minutes) and some previous permissible exposure limits (PEL) based on eight-hour time-weighted average (TWA) standards. Also, individuals who performed this type of work in the past may have had exposures higher than previously suspected. The results demonstrated that employees who remove dry asbestos-containing gaskets with no localized ventilation should wear a full face supplied air respirator with a HEPA escape canister and the work area should be designated a regulated area.

Keywords Asbestos, Gasket, Removal, Exposure

Asbestos-containing sheet gaskets have been used in almost every type of industry for the last 60 years. These gaskets had the ability to prevent leakage between different types of couplings, particularly at elevated temperature and pressure.[1] These types of gaskets normally contained 70 percent to 80 percent chrysotile asbestos by weight. In some cases crocidolite asbestos was used for special applications, that is, sealing flanges in acid lines. The remaining non-asbestos component of the gasket was usually constructed of synthetic rubber material that consisted of either neoprene, styrene butadiene rubber (SBR), or a nitrile polymer.[2–4]

Most companies replaced asbestos fibers in their gasket products with other nonmineral fibers in the late 1980s or early 1990s. This coincided with the Environmental Protection Agency's (EPA) 1989 ban on the manufacture, importation, processing, and distribution of these types of products.[5] However, the United States Fifth Circuit Court of Appeals vacated most of the asbestos ban and Phase Out Rule and remanded it back to EPA in October 1991. Although the court vacated and remanded most of the rule, it left intact the portion that regulated asbestos products that were not being manufactured, produced, or imported when the rule was published in December 1989. Since asbestos-containing sheet gaskets were still being imported into this country, they were exempt from the ban and can still be manufactured, purchased, and used in the United States.

Fowler recently described the problem with the use of these products when he demonstrated that the application of asbestos-containing gaskets had the potential to release respirable asbestos fibers well above current OSHA standards. Fowler recommended that these products should not be used in today's industry and that only non-asbestos gaskets should be used in their place.[6]

An issue that faces many former industrial workers is the past use of these types of gaskets. Workers were not informed in most cases that the products they were using had the potential to release elevated levels of respirable asbestos fibers. Legal issues concerning past exposures pose this basic question: Did handling and performing maintenance activities on these gaskets contribute to their asbestos exposure history? Industrial hygienists must rely on a retrospective exposure assessment to make

this determination.[7] In this approach the individual's work history is compared to the results of retrospective exposure assessment studies that replicate their work activities.

A review of the peer-reviewed literature found very few published studies involving exposure assessments during the dry removal of asbestos sheet gaskets from flanges.[7–9] The studies of Cheng, Millette, and McKinery were somewhat limited in the information reported. Millette used only a small number of flanges. Cheng's work did not verify that all the gaskets contained asbestos. Additionally, there was only limited information provided in all three studies concerning the size and the history of the flanges used or the length of time required for the gasket removal process.

The most comprehensive study to date was by Spence et al.[10] However, the authors used wetting to control the airborne release of asbestos fibers. This limited the study's value for any retrospective exposure assessment since dust control methods were not used in the workplace historically.

In contrast to the previous studies, the goal of these new work practice studies was to estimate a worker's asbestos fiber exposure during the removal of asbestos-containing sheet gaskets using common removal techniques such as scraping, hand wire brushing, and power wire brushing. The studies were conducted on a large population of steam line flanges and valve assemblies. The compilation of several studies discussed in this article allows a more accurate retrospective exposure assessment for individuals who worked with these products in the past and the assessment of potential exposure to workers who may be removing asbestos-containing gaskets today using these same work practices.

High-intensity lighting and videotaping techniques were used inside an exposure characterization laboratory (ECL) during the work practice studies to visually document the pathway of exposure during the gasket removal process and to help determine what activities produce the airborne asbestos dust.

The methods and procedures described in this report can be applied to assessing past and present industrial hygiene exposures to other dusts, fumes, and fibers besides asbestos. The videotaping of dust, fume, and fiber exposures under high-intensity light can be used as a training tool in visualizing the importance and effectiveness of engineering and administrative controls and respiratory protection.

MATERIALS AND METHODS

A number of valve and flange assemblies were collected in 1994 from a paper mill powerhouse in Oregon and stored under ambient conditions in a protective environment until their use in these studies. A sampling of these flange and valve assemblies was partially opened to confirm the presence of asbestos in the sheet gaskets using polarized light microscopy (PLM) prior to the work practice study.[11] Any opened flanges were reassembled and the outside surfaces of all the flanges were cleaned, sand blasted, and repainted. Interviews with former machinists and pipefitters determined that the most common techniques for removing gasket material tightly adhered to the flange surface were hand scraping, hand wire brushing, and/or electric wire brushing.

The work practice simulations were conducted inside an exposure characterization laboratory (ECL) that was constructed as a containment area to prevent the release of asbestos to the outside environment. The dimensions of this containment area were 6.0 m (length) × 4.5 m (width) × 2.4 m (height). The ECL also contained two viewing ports for videotaping purposes and had a decontamination area for contaminated clothing disposal, an air lock for sample removal, and showers to further control fugitive emissions.

Fresh air was produced by a high efficiency particulate absolute (HEPA) filtered negative air machine manufactured by Aramsco (model #550I 1) and pulled through the ECL at a ventilation rate of 5.7 cubic meters per minute. This unit was operated at an air exchange rate of five times per hour (ACH) during the work practice studies. The air in the chamber was flushed between studies by increasing the fresh air ventilation to 28.3 cubic meters per minute for a minimum of 24 hours. At the end of the first scraping and hand wire brushing study (Study 1), the ECL was completely decontaminated by HEPA vacuuming all dust and debris and then wet wiping. Also, all inside surfaces were repainted after the decontamination procedure.

High-intensity lighting (700–1000 watts) was used inside the chamber during videotaping of the work practice to document dust generated by various tasks and to observe pathways of exposure to respirable dust. In previous studies the use of high-intensity lighting was found to be an effective tool to display respirable airborne dust released from asbestos-containing products during work activities.[12,13] The authors performed these studies wearing normal work clothes over disposable protective suits and were equipped with supplied air respiratory protection with HEPA escape filters.

Personal and area air samples were collected during the studies using nonconductive three-piece cassettes. The cassettes contained mixed cellulose ester (MCE) filters that were 25 millimeters in diameter and had a 0.8 micrometer pore size. These filters rested on a MCE backing filter (5.0 micrometer pores). The personal and area air sampling pumps were calibrated before and after the completion of each study against a DryCal primary flow meter to air flow rates of two and ten liters per minute, respectively. High-volume air-sampling pumps (Dawson 110 volt) were used for collecting area air samples during the studies. Four area samples were located in four equidistant quadrants at a distance of 2.1 meters from a work bench placed in the center of the ECL. The area sample cassettes were placed on sampling stands at a height of 1.5 meters. The four calibrated high-volume air sampling pumps were placed outside the chamber and each pump was connected to an area air cassette by Tygon tubing passing through the wall of the ECL.

The two investigators performing the studies were each fitted with two calibrated personal GilAir air sampling pumps with the air-sampling cassettes attached to each shoulder and within their

breathing zones. Background area samples were collected inside and outside the ECL before each study. The air samples were collected in general accordance with the NIOSH 7400 method entitled, "Asbestos and Other Fibers by PCM."[14] Two air sampling cassettes were opened for 30 seconds inside the ECL to serve as personal field blanks at the end of each study.

Surface morphology of new and used gasket material was examined using a Hitachi S-800 field emission scanning electron microscope (SEM). Photomicrographs were taken of the gasket surfaces to document the degree of gasket degradation and the relative amount of asbestos fibers present on the surface.

Study 1—Scraping and Hand Wire Brushing of Small Flange Assemblies

Seven small flange assemblies were used in this study. The gaskets had outside diameters of approximately 69 mm and working widths of approximately 19 mm. Gaskets were removed from one flange on the first four valve assemblies and then from two flanges on each side of the remaining three valve assemblies for a total of ten gaskets. The flange assemblies were first opened and then the gaskets were scraped using a stiff, four-inch-wide putty knife. Any residual gasket material that could not be removed from the flange faces by scraping was removed by hand wire brushing. Some of the gaskets required repetitive scraping and wire brushing to remove the gasket and to polish the flange face. The sheet gaskets were removed sequentially from each of the 10 flanges.

One of the investigators in the ECL simulated the worker who did all of the gasket removal while the other acted as a "helper." The helper changed the area and personal air sample cassettes periodically throughout the study. Each gasket was collected and retained for analysis to determine both asbestos content and matrix identification after removal. The investigators were in the ECL for 194 minutes. All air sample cassettes in the ECL were exchanged every 15 to 30 minutes. A total of seven sets of air samples were collected.

Study 2—Scraping and Hand Wire Brushing of Large Flange Assemblies

Four large flange assemblies were used for this study. The outside diameter of these gaskets varied from 125 mm to 200 mm and the gaskets were 19 mm to 25 mm wide. The gaskets were removed and collected from the four flanges as described in Study 1. The investigators were in the ECL for 113 minutes. All air sample cassettes in the ECL were exchanged every 15 to 30 minutes. A total of five sets of air samples were taken during this work practice simulation.

Study 3—Power Wire Brushing of Large Flange Assembly

An electric wire brush (Skil electric drill 0.3 Hp with a Columbian 10.2 cm crimped wire wheel) was used during this study to remove gasket residue that could not be removed during the scraping and hand wire brushing of the first flange assembly used in Study 2. The electric wire brush was also used to polish the flange face surfaces. This study was conducted one day after Study 2. The ECL was not decontaminated between the studies. The two flange surfaces were electric wire brushed until the gasket residue was visibly removed. As previously described in Study 1, the two investigators were in the ECL performing the study.

One person did the removal work while the other assisted as the helper. The residual gasket material was not retained since the bulk of the material was collected in Study 2. The investigators were in the ECL for 42 minutes. The air cassettes in the ECL were exchanged every 10 minutes. A total of four sets of air samples were taken during the electric wire brushing activity.

All air filters collected were analyzed by PCM in general accordance with the NIOSH 7400 method using the "A" counting rules. Additionally, all air samples were prepared for TEM examination using the indirect preparation method.[15] The indirect TEM preparation method was chosen because filter overloading rendered the samples unsuitable for direct preparation despite frequent changing of the air sample cassettes. Also, the indirect TEM preparation method enabled data comparisons to other published and unpublished studies previously performed that also used the indirect TEM method.[16–18] The TEM air samples were then analyzed by a modified EPA Level II protocol.[19] Cloth swatches from the work clothing worn by the investigators during the studies were analyzed by the recommended EPA method.[20] Surface dust samples were collected from the work table after each gasket removal study and analyzed according to the ASTM protocol.[15] Background samples from the clothing and the work table surface were also collected before each study was started. The removed gaskets were analyzed for asbestos type and content by the standard PLM method.[11]

RESULTS

It was determined by PLM that the gaskets removed in these studies contained 65 percent to 85 percent chrysotile asbestos (Table I). Table II and Table III, respectively, illustrate the PCM and TEM results for Study 1. The worker in Study 1 had a peak exposure level of 10.1 fibers per cubic centimeter (f/cc) and an 8-hour TWA exposure of 1.5 f/cc. The area air samples were voided after the completion of Study 1 when it was determined that the air-sampling lines into the ECL were obstructed. The

TABLE I
PLM analysis of removed gaskets

Studies	Number of gaskets analyzed	Asbestos type	Concentration of asbestos in volume percent
Study 1	10	Chrysotile	65–80%
Study 2	4	Chrysotile	75–85%
Study 3	1	Chrysotile	85%

TABLE II

Study 1—Scraping and hand wire brushing: small flanges. PCM airborne exposure levels (fibers greater than 5 micrometers)

Sample type	No. of air samples analyzed	Range (f/cc)	Sample time weighed average (f/cc)	8-hr TWA (f/cc)
Background	4	0.0	0.0	N/A
Worker	14	1.5–10.1	3.7	1.5
Assistant	14	1.2–4.2	2.4	1.0
Area samples[A]	36	—	—	—

Total air-sampling time = 194 minutes.
[A]The air-sampling lines into the ECL were obstructed, voiding the area air samples in this study.

results for Study 2 are shown in Tables IV and V. The worker in this study had a peak exposure level of 24.0 f/cc and an 8-hour TWA of 3.6 f/cc. Table VI and Table VII list results for Study 3. The peak exposure level found while power wire brushing was 31.0 f/cc and the calculated 8-hour TWA was 2.3 f/cc. The results for the surface dust samples taken from the work table and the fabric samples are shown in Table VIII. All PCM and TEM data in the tables are expressed for comparison purposes as fibers per cubic centimeter (f/cc) greater than 5.0 micrometers in length.

DISCUSSION

The asbestos concentrations measured in these studies were higher on average than other previously published studies for similar work practices.[7–9] It is believed that the higher concentrations found in these studies were due largely to the gaskets adhering more tightly to the flanges. Tightly adhered gaskets require higher energy for removal. As described by Fowler, the friability of the product is always relative to the energy applied.[6] Only two of the fourteen gaskets removed could have been described as easily detached. The other twelve required extensive effort on one or both of the flange faces. Machinists, pipefitters, steamfitters, and others commonly described sheet gaskets as tightly adhering to flange surfaces and requiring substantial work to remove the gasket material. Unfortunately, the various conditions and the amount of adhesion of the gaskets in the previously published studies were not reported.[7–9] The adhesion of gasket materials generally has been related to its length in service and the conditions of service such as temperature and pressure. The high temperature steam flanges used in this study were from a steam powerhouse that operated for a number of years. The last steamfitter who maintained the steam system indicated that gasket replacement was rare due to infrequent plant downtime and few leaks. Gaskets that could be easily removed would not be expected to produce airborne levels comparable to what was found in these studies. None of the previous studies described the level of difficulty of removing the gaskets from the flange surfaces.

TABLE III

Study 1—Scraping and hand wire brushing: small flanges. TEM airborne exposure levels (asbestos fibers greater than 5 micrometers)

Sample type	No. of air samples analyzed	Range (fibers/cc)
Background	4	0.0
Worker	14	29.9–144.2
Assistant	14	2.2–29.5

Total air-sampling time = 194 minutes.

The air samples collected were analyzed by both PCM and TEM during the gasket removal activities in these studies. The two basic types of sample preparation for TEM air analysis are the direct and indirect methods.[15,16,21–23] Some scientists have suggested that the indirect sample preparation method, particularly the sonication step, causes large complex asbestos structures such as fiber bundles and clusters to break up and bias fiber counts to higher concentrations.[24,25] However, studies performed by the EPA and others have shown that this criticism is not valid and that the indirect technique is an acceptable method to analyze overloaded air samples.[26–28]

The overloading of other particulates on an air filter will obscure fibers that are collected. This condition can lead to the undercounting of asbestos fibers if a direct preparation method is used. Controlling the particulate loading on a filter can be difficult when the disturbance of materials generates large amounts of both fibrous and nonfibrous airborne particulates. The general approach to reduce or eliminate overloading conditions is to alter flow rates and sampling times. However, particulate loading can be controlled by using the indirect preparation method without compromising sampling times. The overloading problem can also affect the direct examination of air filter samples by PCM (NIOSH 7400 method). This was noted in Study 1. The asbestos air concentrations measured by PCM in Study 1 decreased as the study progressed. This would not be consistent with the continued activities that took place inside the ECL during the study. This effect was due to particulate overloading on the filters. However, according to the TEM data from Study 1, the asbestos fiber concentrations tended to increase as the work progressed. The sampling times for Studies 2 and 3 were reduced in an effort to minimize overloading on the PCM air samples.

TABLE IV

Study 2—Scraping and hand wire brushing: large flanges. PCM airborne exposure levels (fibers greater than 5 micrometers)

Sample type	No. of air samples analyzed	Range (f/cc)	Sample time-weighted average (f/cc)	8-hr TWA (f/cc)
Background	4	0.0	0.0	N/A
Worker	10	9.3–24.0	15.3	3.6
Assistant	10	5.2–15.7	8.8	2.0
Area samples[A]	24	2.1–8.4	—	—

Total air-sampling time = 113 minutes.
[A]TWA not calculated for area or "bystander" samples.

However, any further reduction in the sampling time would have had an impact on the work activities. Therefore, the air-sampling times were not decreased any further.

The current OSHA asbestos exposure standards are based on the NIOSH 7400 method. This method measures only fibers longer than 5 micrometers in length and greater than 0.25 micrometers in width. However, these fiber dimensions were not implemented by OSHA with regard to health issues. The minimum dimensions were implemented solely due to the fiber resolution limitations of the PCM technique.[29] OSHA has long recognized that PCM is not fiber-specific or able to resolve fibers that are less than 0.25 micrometers in width. The TEM analysis performed in these studies augmented the PCM measurements by obtaining more complete and accurate measurements of the airborne asbestos concentrations.

A comparison of the air data collected from the PCM and TEM analyses showed fiber concentrations approximately 30 times greater in the TEM analysis. The differences between TEM and PCM measurements have been recognized by others and are primarily due to the resolution limitations of the optical microscope.[30,31] The deficiencies of PCM measurements are especially acute when products such as sheet gasket materials that contain high percentages of chrysotile fibers are the source of the airborne fibers. It has been shown that free respirable chrysotile fibers are released when asbestos-containing products are abraded in some manner.[6]

Work by the EPA demonstrated that single chrysotile fibers have an average diameter of between 0.03 and 0.07 micrometers.[32] This average diameter is approximately five times below the resolution of a phase contrast microscope. Therefore, single chrysotile fibers cannot be seen or counted using the PCM method, irrespective of their lengths. Because of the inherent errors in PCM analysis, it was suggested by the director of the Health Effects Institute for Asbestos Research that OSHA should consider changing to TEM air sample analyses for occupational workplace compliance to adequately protect workers' health.[33]

TABLE V

Scraping and hand brushing: large flanges. TEM airborne exposure levels (asbestos fibers greater than 5 micrometers)

Sample type	No. of air samples analyzed	Range (fibers/cc)
Background	4	0.0
Worker	14	199.6–842.7
Assistant	14	13.6–101.0
Area samples	24	3.3–108.8

Total air-sampling time = 113 minutes.

An SEM examination of the sheet gaskets was performed to better understand the relationship between the physical activity of removal and the measured asbestos air levels found in this study. Generally, sheet gaskets are comprised of approximately 70 percent chrysotile asbestos bundles in a synthetic rubber matrix. The SEM micrograph (Figure 1) shows large bundles of asbestos protruding from the matrix of new sheet gasket material. Any minimal disturbance or abrasion of these bundles can release asbestos fibers into the air. Another problem with asbestos gaskets is that the synthetic rubber matrix begins to deteriorate after installation. In most cases installed sheet gaskets are subjected to high temperature and pressure that will increase the rate of thermal decomposition of the rubber matrix. This produces cross-linking of the polymer molecules. The cross-linking process increases the gasket material's friability by causing the rubber matrix to degrade and become brittle.[34]

A comparison of the surface of a new gasket (Figure 1) to that of a used gasket removed from one of the flanges in Study 2 (Figure 2) demonstrates how the rubber matrix material is degraded. This degradation provides more opportunity for the release of asbestos fibers during the removal process. The fiber concentrations measured in Study 2 were higher than those measured in Study 1 even though more gaskets were removed in the first study. Factors believed to lead to these results were as follows: (1) The total gasket surface area removed in Study 2 was much larger than in Study 1, (2) The gaskets in Study 2 were observed to be more friable and more deteriorated, and (3) All the gaskets in Study 2 tore apart and remained adhered or attached to both of the flange faces when the flanges were opened.

An electric powered drill equipped with a wire brush was used to remove some residual gasket material from two flange faces in Study 3. The resulting exposures during the work activities

TABLE VI
Study 3—Power wire brushing. PCM airborne exposure levels (fibers greater than 5 micrometers)

Sample type	No. of air samples analyzed	Range (f/cc)	Sample time-weighted average (f/cc)	8-hr TWA (f/cc)
Background	4	0.09–0.12	0.11	N/A
Worker	7	14.9–31.0	21.8	2.3
Assistant	8	12.8–21.2	15.9	2.0
Area samples[A]	16	7.6–15.7	—	—

Total air-sampling time = 42 minutes.
[A]TWA not calculated for area or "bystander" samples.

were higher even though the residual gasket material was far less than the gasket materials removed in Study 1 and Study 2. It was observed in Study 3 that the mechanical action generated from the power wire brush tore loose more asbestos fibers and propelled them greater distances into the air. This observation supported the higher asbestos air concentrations of the area samples measured in Study 3 compared to those measured in Study 2. The results from the surface dust and fabric samples (Table VIII) showed that the surface asbestos levels measured can be classified as "highly contaminated" and pose additional exposure problems to the worker throughout the workday. Additional asbestos exposure can occur to both the worker and other family members if the clothes are worn away from the job or taken home.[35]

CONCLUSIONS AND RECOMMENDATIONS

These studies, as well as the other studies previously discussed, demonstrate that there can be wide variability in airborne asbestos fiber levels generated during the removal of asbestos-containing gaskets from flanges. The variability of fiber levels released is most likely dependent on the condition of the asbestos gasket, the size of the gasket surface area and the method of removal. The condition to which a gasket is subjected determines the degree of adhesion of the gasket to the flange surface and the friability of the gasket. This impacts the amount of energy required to remove the gasket and release asbestos fibers. The determining factors that seem to affect the condition of the gasket are: length of service, temperature and pressure conditions, and composition of the gasket matrix.

Our data show that dry removal methods typically used by machinists and pipefitters (past and present) result in significant airborne asbestos fiber exposures. For retrospective asbestos exposure assessments, the exposures measured by PCM in Studies 1, 2, and 3 exceed all historical OSHA excursion limits and some previous permissible exposure limits (PEL) based on an eight-hour TWA. The exposures also far exceed current OSHA levels. Therefore, former machinists and pipefitters that performed this type of work as part of their job activities would have had significant airborne asbestos exposures when removing tightly adhered gaskets on flange surfaces.

Under normal lighting, airborne dust is invisible even though the asbestos levels measured are above OSHA excursion limits. Therefore, an individual removing asbestos-containing gaskets will be unaware of any airborne exposure problems under normal working conditions. High-intensity lighting (Tyndall Effect) was used by the investigators in these studies to observe exposure mechanisms for workers performing normal work activities. The Tyndall Effect documented fiber release mechanisms and the pathways of exposure to the individuals removing the gaskets. Tyndall lighting is an alternative technique that industrial hygienists can use to check potential airborne dust emissions in the workplace. The Tyndall lighting technique can visually demonstrate to workers and employers if there is a need for air sampling, additional ventilation, respiratory protection, and/or special work practices.

There are still significant numbers of asbestos gaskets currently being used in the United States. OSHA classifies the

TABLE VII
Study 3—Power wire brushing. TEM airborne exposure levels (asbestos fibers greater than 5 micrometers)

Sample type	No. of air samples analyzed	Range-fibers/cc
Background	4	0.0–0.2
Worker	7	877.1–1636.1
Assistant	8	60.4–364.4
Area samples	16	56.9–801.9

Total air-sampling time = 42 minutes.

TABLE VIII
TEM fabric and surface dust contamination levels

Studies	Fabric-fibers/cm^2	Surface dust-fibers/cm^2
Study 1	981 thousand	8.5 million
Study 2	3.2 million	27.8 million
Study 3	19.3 million	57.4 million

All background control samples and field blanks analyzed were below the analytical detection limit.

FIGURE 1
Scanning electron micrograph of the surface of a new asbestos-containing gasket. Both the chrysotile fibers and polymer matrix are visible. Magnification 1000×.

FIGURE 2
Scanning electron micrograph of the surface of a used asbestos-containing gasket. The majority of the material present is only chrysotile asbestos. Magnification 1000×.

removal of asbestos-containing gaskets as Class II work of short duration.[36] This specification by OSHA only addresses a single gasket removal project. However, interviews with pipefitters and machinists indicate that only removing one gasket at a time was not a typical occurrence. Under current OSHA regulations, the removal of asbestos-containing gaskets requires the use of a glove bag and wetting methods to contain the release of asbestos fibers into the workplace. Unfortunately, the glove bag and wetting methods are not always practical in an actual workplace due to production and maintenance schedule pressures and the difficulty in wetting a rubber based gasket.

The results of these studies indicate that employers need to determine if asbestos-containing gaskets are present in their equipment. The employer must immediately comply with OSHA's Class II provisions by implementing a safe operating procedure that includes employee training, assessment/monitoring, containment, and good work practices. The following actions are recommended if asbestos-containing gaskets are removed without a glove bag and wetting: (1) A negative pressure enclosure should be used, (2) The enclosure should have a HEPA filtering/air blower system, (3) A HEPA vacuum cleaner and wetting agents should be used, and (4) The worker should wear a respirator appropriate for the airborne asbestos concentrations generated by the activities.

The data presented here demonstrate that the work surfaces in these studies as well as the clothing worn by the investigators were highly contaminated with asbestos fibers. An asbestos-contaminated workplace can lead to additional asbestos exposures. The disturbance of the dust around the work area by other work activities and housekeeping activities will re-entrain asbestos fibers into the air.[35] The wearing, changing, and washing of the contaminated clothing can also lead to asbestos exposures for both a worker and family members.

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Exhibit 16

Zonolite Attic Insulation Exposure Studies

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Several studies were designed and conducted to evaluate amphibole asbestos exposures in homes containing Zonolite (expanded vermiculite) attic insulation (ZAI). A range of tasks selected for evaluation included cleaning, working around, moving, and removal of ZAI in attics and living spaces. The fieldwork for these studies was conducted at two homes in Spokane, WA and one home in Silver Spring, MD. Personal and area air samples were collected and analyzed as part of the exposure studies. Surface dust samples and bulk samples were also collected and analyzed. The results demonstrated that airborne concentrations of amphibole asbestos were not elevated if the material is undisturbed. The results also demonstrated that cleaning, remodeling, and other activities did produce significant concentrations of airborne amphibole asbestos when the ZAI was disturbed. *Key words*: asbestos; vermiculite; amphibole; exposure; insulation; renovation; remodeling; demolition; industrial hygiene; Zonolite; ZAI.

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INTRODUCTION

In 1926, the Vermiculite and Asbestos Company was formed to extract vermiculite from the Libby, MT area; since the time of the company's formation, it was known that vermiculite from Libby was contaminated with asbestos.[1] Two years later, on November 27, 1928, US patent number 1,693,015 was awarded to Joseph A. Babor and William L. Estabrooke for a molded insulating material made from expanded vermiculite, termed Zonolite.[2] One of the major uses of Zonolite was loose-fill insulation in attics of homes. By 1977 such loose-fill insulation, or Zonolite Attic Insulation (ZAI), constituted 15 % of domestic vermiculite use.[3] During each year of the 1970s alone approximately 53,000 tons of vermiculite were installed into US homes, according to a study commissioned by the United States Environmental Protection Agency (EPA).[4] The mines in Libby were the largest source of this vermiculite.[3]

Over the decades, studies were done at the Libby mine and mill as well as at other industrial sites evaluating exposures for asbestos-contaminated vermiculite.[5] Studies have also been performed, and ongoing studies are evaluating, past and current exposures to amphibole asbestos and resulting disease in the Libby area and numerous expansion plants.[6,7] W.R. Grace & Co. (WRG) produced and sold ZAI for many years. The company no longer produces ZAI and has filed for bankruptcy. The scientific and medical literature includes thousands of articles evaluating asbestos exposure and disease in asbestos mining and milling operations, asbestos product manufacturing and installation, and asbestos abatement. There is a small collection of articles that consider asbestos exposure and disease from fibers carried into the home from the workplace. Other studies have looked at concentrations of asbestos in the outdoor air, and some have summarized air sampling measurements inside public and commercial buildings. People are clearly exposed to airborne contaminants not only in the workplace but in the outdoors as well. However, many, if not most people spend more time in their home environment than any other and, significantly, there is a gap in the literature when considering asbestos exposure from materials in the home. In this study we looked at amphibole asbestos exposure in homes from attic insulation made from expanded vermiculite, or ZAI.

The first study to report exposures from disturbing in-place asbestos-contaminated vermiculite was presented at the American Industrial Hygiene Conference in 1997.[8] This study measured exposures to workers when demolishing a building with asbestos-contaminated attic insulation in Manitoba, Canada.[9] Samples of the vermiculite attic insulation were reported as containing generally less than 0.1% actinolite and/or tremolite asbestos. This study reported personal exposures to workers demolishing a ceiling, performing clean-up, and disposing of the waste, which ranged from 3.3 to 6.8 fibers greater than 5 µm in length per cubic centimeter (f/cc). The same samples analyzed by transmission electron microscopy (TEM) found 4.4 to 174 asbestos fibers greater than 5 µm per cubic centimeter (f/cc). This study did not address what expo-

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Disclosures: The field study and laboratory analyses for this work were funded by attorneys representing claimants in the W.R. Grace & Co. bankruptcy proceedings. W.R. Grace & Co. formerly manufactured Zonolite expanded vermiculite attic insulation (ZAI) for use in homes. The authors have previously appeared as expert witnesses in asbestos litigation on behalf of building owners against former asbestos product manufacturers.

Figure 1—Home A.

Figure 2—Home B.

Figure 3—Home C.

sures, if any, might result from routine tasks performed by homeowners in attics with Zonolite vermiculite.

We designed and conducted a series of studies to evaluate amphibole asbestos exposures during specific activities conducted in homes containing ZAI. The tasks selected for evaluation were as follows:

- cleaning stored items in an attic with ZAI at the perimeter only;
- cleaning storage areas in an attic fully insulated with ZAI;
- cutting a hole in the ceiling of a living space below ZAI attic insulation;
- moving ZAI using the WRG method;
- moving ZAI using a homeowner method; and
- removing ZAI from the top of wall cavities with a shop vacuum.

METHODS

Selection of Homes

One of the authors visited over a dozen homes to determine if they were possible candidates. The primary criterion was the presence of Zonolite vermiculite used as insulation in the home. The homes also needed to be available for study and sampling over approximately a three- to four-day period. The testing was designed to avoid exposing the occupants to any additional asbestos. The homes selected needed to have reasonable access to the attics. The availability of electricity and water was also necessary. Three homes were selected (Figures 1, 2, and 3).

Selection of Tasks

Possible activities during which asbestos exposures might be measured were considered during preparation of the study design. These included cleaning tasks, service work, maintenance, remodeling, renovation, and demolition activities. The category "no activity" was considered and selected as a baseline for comparison with the tasks to be tested. Long-term sampling in occupied homes was not considered feasible due to time and budgetary constraints. Tasks selected for testing were those that might occur in homes and that might reasonably be expected to disturb in-place Zonolite insulation or the dust/debris from that insulation.

Description of Tasks

Before conducting testing, the area where each task would occur was separated from the rest of the house by erecting a two-stage decontamination station at the entrance to the attic or room. Each decontamination station consisted of two small rooms (approximately 4′ × 4′) separated by plastic flap doorways and was similar to those used on asbestos abatement projects. The inlet for a high efficiency particulate air (HEPA) filtered vacuum was placed in the room closest to the work area. The decontamination station was designed to prevent dust generated from the activities conducted from migrating out of the attic or room. It also served as a location for persons to change out of personal protective equipment and to clean themselves and equipment. As necessary, suspended shop lights were installed to provide better lighting. Area sampling equipment, extension cords, tripods, and miscellaneous tools/sup-

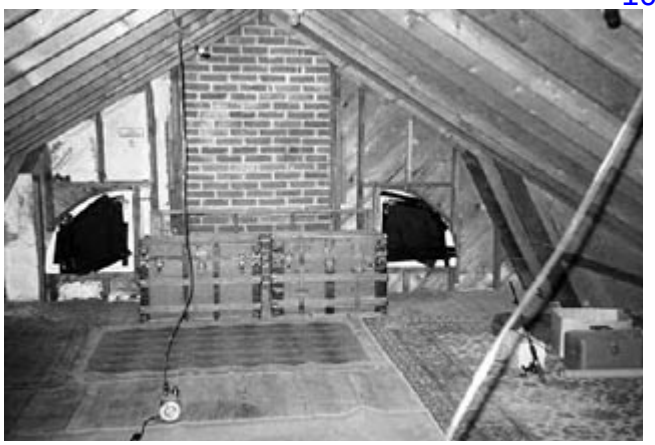

Figure 4—View of attic area cleaned in home B.

Figure 5—View of attic in home C.

plies necessary to perform the tasks were brought into the area.

After the tasks were performed, any items removed from the area were HEPA-vacuumed and wet-cleaned. Accessible Zonolite insulation in the attics of the homes was removed by a state licensed asbestos abatement contractor. During and after these activities, area air sampling was conducted by a local consulting firm to determine if asbestos had migrated to normally occupied locations and if the attics were clean after abatement.

Cleaning of stored items in an attic with Zonolite at the top of wall cavities only. This activity was performed in the attic of home B (Figure 4). In this home the Zonolite insulation was limited only to the perimeter (primarily the east and west sides) of the attic space at the top of the wall cavities. Cleaning was performed by one individual with an assistant to help move trunks and boxes.

The cleaning consisted of dusting the top surfaces of approximately eight stored boxes, two trunks, and fishing tackle with new cotton cloths, as well as sweeping exposed wood floor areas with a corn broom (Harper brand, model No. 100, Harper Brush Works, Fairfield, IA 52556). Rugs on the attic floor were cleaned with a standard upright vacuum cleaner (Eureka brand Upright Vacuum Cleaner, Household Type, Model No. 7600, The Eureka Company, Bloomington, IL 61710). The homeowner reported the attic had last been cleaned two years prior to this work and we followed the procedures in the same manner as that cleaning, as described by the homeowner. About half of the attic floor area was cleaned (approximately 390 ft^2). The cleaning activity took 31 minutes to complete and were completed in the following order: sweeping (1 min) dusting (13 min), and vacuuming (17 min).

Cleaning of storage area in an attic fully insulated with Zonolite. This activity was performed by one person in home C, who used a new corn broom to sweep spilled ZAI back into the space between ceiling joists in the attic (Figure 5). The person also used a hand broom to sweep ZAI from wooden boards located in the attic. The task took approximately 16 minutes to complete.

Cutting a hole in the ceiling of a living space below Zonolite attic insulation. This activity was performed at home A (Figure 6). The hole was similar to one that might be needed to install a recessed light fixture or ceiling fan. One person cut an opening in the ceiling measuring 15″ × 24″ in a room measuring 11′2″ × 13′4″ with the assistance of a second person. The ceiling material consisted of a stipple finish on 1/4″ wallboard, one layer of wallpaper, finish hard plaster, and a coating of gray hard plaster on wood lathe.

The cutting was started by drilling a 2″ diameter hole at one corner of the rectangle to be cut with a power drill equipped with a keyhole saw bit. The remainder of the cutting was performed with a Stanley brand 12″ hand compass saw (both the keyhole and the compass saw had eight-point blades). The entire cutting activity took 24 minutes to complete with drilling the starting hole taking less than one minute and the remainder of the time spent hand-sawing with periodic short rest breaks. The average depth of Zonolite insulation above the cutout area was 4″.

Moving aside Zonolite attic insulation (W.R. Grace & Co. method).[10] This activity was performed in the attic of home A (Figure 7). The floor of the attic was 756 ft^2 (28′ × 27′). This task was performed primarily by one person with the assistance of a second person.

The activity consisted of removing approximately 15 ft^2 (2′6″ × 6′) of ZAI having an average depth of 5″ from between the floor joists. This material was misted with water using a hand-held pump-up garden sprayer immediately before the work began. The Zonolite was scooped from between the floor joists and into plastic bags using a plastic dustpan. The remaining visible dust and debris was removed using a new HEPA-filtered vacuum cleaner (Ridgid brand, model no. WD09350, manufactured by Emerson Electric Co., with a Trapmax 3 model no. VF6000 HEPA filter rated at 99.97% efficient down to 0.3 microns installed).

Figure 6—View of ceiling after cutting, home A.

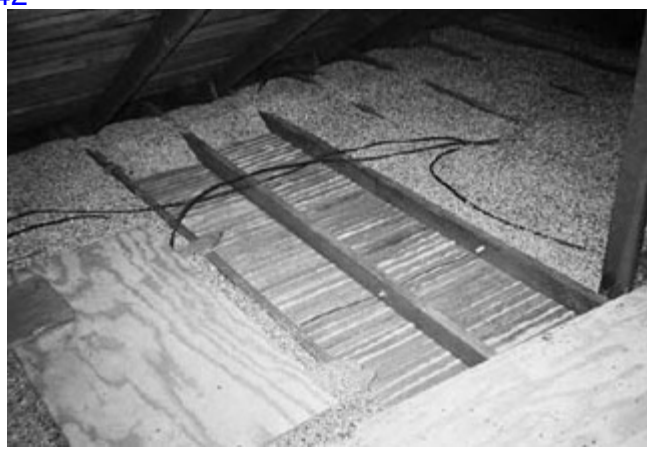

Figure 7—View of ZAI after moving by W.R. Grace & Co. method.

The activity took 33 minutes to complete, consisting of two minutes for misting with water, 25 minutes for scooping Zonolite into plastic bags, and six minutes for vacuuming.

Moving aside Zonolite attic insulation (homeowner method). This task was performed in the same attic (home A) as the previous test. This activity consisted of removing approximately 14.4 ft^2 (2′8″ × 5′5″) of Zonolite attic insulation with an average depth of 5″ from between the floor joists (Figure 8). The work was performed using the same methods, except the Zonolite was not misted with water at the start of the work and a whiskbroom and plastic dustpan were used to remove the visible dust and debris at the end of the work (O Cedar brand corn whiskbroom, 10″ long, bristle spread 8″ by 1″). The work took 29 minutes to complete, consisting of 15 minutes scooping ZAI into plastic bags and 14 minutes using a whiskbroom to clean dust and debris.

Removal of Zonolite insulation from the top of wall cavities with a shop vacuum. This activity was performed in the attic of home B (Figure 9). The removal was performed by one individual with an assistant. The work consisted of removing approximately 60′ of Zonolite insulation from a trough at the perimeter of the attic having an average width of 5.5″ and depth of approximately 4″. The equipment used to remove the Zonolite was a new standard shop vacuum (Ridgid brand, model no. WD0620, manufactured by Emerson Electric Co., with part no. VF4000 filter installed). The work took 44 minutes to complete and consisted of vacuuming up Zonolite until the shop vacuum was about half full (approximately three gallons) and dumping the contents into a plastic trash bag. The shop vacuum was emptied seven times during this activity.

Personnel Protection

Prior to the start of any field work, and again at the work sites, all personnel were briefed on the project and the known health and safety hazards likely to be encountered. During the testing, any persons entering the attics or other work areas were required to wear respiratory protection and two layers of full body protective clothing. Full-face powered-air purifying respirators equipped with high efficiency filters approved by the National Institute for Occupational Health and Safety (NIOSH) to prevent asbestos exposure were used. Personnel decontamination was performed on-site through the use of a HEPA-filtered vacuum followed by wet washing. Homeowners were not permitted to enter the home until after cleaning was completed by a state licensed asbestos abatement contractor and clearance air sampling had been completed.

Sampling Methods

Air, dust, and bulk samples were collected as part of this study. Sample logs and chain-of-custody forms were completed for all samples. Air, dust, and bulk samples were stored and transported separately to minimize the opportunity of cross-contamination between samples. The amphibole asbestos species identified by electron microscopy or polarized light microscopy in air, dust, or bulk samples are reported herein as "Libby amphiboles" and consisted of fibrous tremolite, richterite, winchite, and actinolite.[11,12]

Air sampling. Personal and area air sampling was conducted. Personal samples were collected in the breathing zone of the person, but outside the full-face respirator. The personal samples were secured to the full-face respirator at approximately eye level so the sample would not be located in the exhaust of the powered-air purifying respirator. The filter cassettes were positioned at approximately a 45-degree angle pointed downward. Personal samples were collected using battery-operated air sampling pumps calibrated before and after each set of samples during an activity (Mine Safety Appliance [MSA] brand model ELF sampling

Figure 8—View of Zonolite in attic after moving by homeowner method.

pumps and one MSA brand model Flowlite pump). Area samples were collected using electric air sampling pumps (Dawson brand Gast electric pumps). All personal sampling pumps were calibrated on-site using a primary flow meter (Bios International Corp., DryCal DC-Lite Primary Flow Meter, S/N 6615).

Personal samples were collected in pairs. One sample was collected on a mixed cellulose ester (MCE) membrane filter (25 mm diameter) having a pore size of 0.8 micrometers (µm). The other sample in the pair was collected on the same type of filter with a pore size of 0.45 µm. Personal samples were typically collected at flowrates between 0.5 and 1.0 liters per minute (l/min) due to the dusty environment anticipated. Area samples were typically collected at flowrates of seven to 10 l/min in less dusty environments and two to four l/min in more dusty environments.

During the testing, the personal and area air sample filters were visually inspected at least every five minutes to estimate dust loading. The sampling filters were changed whenever there was a visible discoloration of the filter surface to reduce the chance of excessive dust loading on the filters. Blank samples were collected at a rate of 10% or two per sampling batch, whichever was greater.

All air samples were submitted to a laboratory accredited by the American Industrial Hygiene Association (AIHA) and the National Voluntary Laboratory Accreditation Program (NVLAP) (administered by the National Institute of Standards and Technology (NIST), or were A2LA accredited under ISO Standard 17025. Personal air samples collected on 0.8 µm pore size MCE filters were analyzed by phase contrast microscopy (PCM) as described in NIOSH method 7400.[13] Personal and area air samples collected on 0.45 µm MCE filters were analyzed by transmission electron microscopy (TEM) using the direct preparation techniques described in the EPA Code of Federal Regulations.[14] This method is commonly referred to as the EPA AHERA method. The results of the PCM samples are reported as fibers per cubic centimeter of air sampled (f/cc). The results of the TEM samples are reported as structures per cubic centimeter of air samples (s/cc). Using the TEM fiber size information for four of the five sets of data, the PCM equivalent (PCME) concentrations were calculated and reported in f/cc.

Dust sampling. Surface dust samples were collected using ASTM method D 5755, Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Concentrations.[15] This method uses a sampling pump calibrated at two l/min to vacuum dust onto a 0.45 µm pore size MCE filter from a measured surface area of typically 100 square centimeters (cm^2). These samples were analyzed by TEM as described in ASTM D 5755 and results reported as asbestos structures per square centimeter of surface area sampled (s/cm^2).

Bulk sampling. Bulk insulation samples were collected by placing a small quantity in a labeled sealed container, and submitted for analysis by polarized light microscopy (PLM) as described by the method EPA-

Figure 9—View of ZAI at top of wall cavity before shop vacuum removal.

TABLE 1 Summary of Air Sampling Results for Cleaning of Stored Items with Zonolite at the top of Perimeter Wall Cavities Only

Sample Location	Number of Samples n	PCM TWA f/cc	TEM TWA s/cc	s/cc >5 µm	PCME (f/cc)
Worker, personal	3,3	1.54	< 0.42	< 0.42	< 0.42
Assistant, personal	3,3	0.53	< 0.33	< 0.33	< 0.33
Area, in cleaning area	3	—	0.12	0.11	0.10
Area, adjacent to cleaning area	3	—	0.07	0.07	0.04
Area, ~10 feet away	3	—	0.06	0.06	0.06
Area, ~20 feet away	3	—	< 0.05	< 0.05	< 0.05
Area, before cleaning	4	—	< 0.002	< 0.002	< 0.002

600/MR-82-020, Interim Method for the Determination of Asbestos in Bulk Insulation Sample.[16] Results are reported as percentages of asbestos by volume. This standard EPA PLM method sometimes fails to detect the amphiboles present in vermiculite samples due to the non-homogeneous distribution of the amphiboles in the vermiculite. Since this work was performed, the EPA has published an improved method designed specifically for analyzing vermiculite-containing attic insulation.[17]

RESULTS

Cleaning of Stored Items in an Attic with Zonolite at Top of Perimeter Wall Cavities Only

Four area air samples were collected before the start of cleaning activities. No asbestos structures were detected in these samples and a detection limit of less than 0.002 s/cc was reported. During the cleaning activity the personal exposure measurements for the worker measured by PCM ranged between 0.82 and 2.53 f/cc, with a time-weighted average (TWA) during the 33-minute time period of 1.54 f/cc. During a 34-minute time period the personal exposure measurements for the assistant measured by PCM ranged between < 0.54 and 0.82 f/cc, with a TWA of 0.53 f/cc. The value of one-half the detection limit value was used to calculate the TWA where no fibers were detected in the sample. To use zero would likely bias the calculated TWA low, and to use the detection limit value would bias the calculated TWA value high. No asbestos structures were detected in three samples collected on the worker and the three samples collected on the assistant during the cleaning activity. The TWA values were < 0.42 s/cc for the worker and < 0.33 s/cc for the assistant.

Four sets of three area air samples (12 total) were collected during the cleaning activity and analyzed by TEM. The TWA during a 31-minute time period for the three samples in the group closest to the cleaning activity was 0.12 s/cc for all structures greater than 0.5 µm in length and 0.11 s/cc for structures > 5 µm in length. The TWA during a 32-minute time period for the next closest set of three area air samples was 0.07 s/cc for structures > 5 µm in length. The TWA during a 32-minute time period for the next closest set of three area air samples was 0.06 s/cc for structures > 5 µm in length. The TWA during a 31-minute time period for the set of three area air samples farthest from the cleaning activity was < 0.05 s/cc. No asbestos structures were detected in these three samples. The results for the air samples collected for this cleaning activity are summarized in Table 1.

Before the cleaning activity began four dust samples were collected from four non-porous attic surfaces. The results ranged from not detected to 38,000 s/cm^2, with an average (logarithmic mean) of 9500 s/cm^2. Three bulk samples of Zonolite collected from the attic perimeter were analyzed by PLM and found to contain a "trace" of Libby amphiboles by volume (a "trace" finding by PLM is an estimate of some value less than 0.1%).

Just prior to the cleaning activity four sheets of aluminum foil were placed on surfaces to collect dust settling during a 23-hour period. The locations ranged from about 10′ to 20′ away from the cleaning activity so they would not need to be disturbed during the cleaning activity. No asbestos structures were found in the four dust samples collected from the foil sheets. Values < 300 s/cm^2 are reported for each sample.

This cleaning study highlights a shortcoming in two commonly used air sampling methods when employed to measure fibers or asbestos structures in a "dusty atmosphere." The direct preparation TEM method requires that small sample volumes be collected to prevent overloading of the filter surface. When the dust collected is predominantly asbestos, this is not a problem. When the dust collected is predominantly not asbestos, the non-asbestos dust obscures the asbestos structures. The result is a higher than desirable sensitivity. For the PCM samples, the non-asbestos fiber content of normal house dust (primarily cellulose fiber) provides for a high fiber count when only a fraction of those fibers are asbestos.

For this study, the three area air samples collected in the cleaning area provided the best asbestos fiber exposure information for an individual cleaning stored

TABLE 2 Summary of Air Sampling Results for Cleaning of Storage Area in an Attic Fully Insulated with Zonolite

Sample Location	Number of Samples n	PCM TWA (f/cc)	TEM TWA (s/cc)	s/cc >5 μm
Worker, personal	3,3	2.87	4.00	2.58
Assistant, personal	3,3	0.65	0.43	0.43
Area, sample set 1	3	—	0.88	0.61
Area, sample set 2	3	—	0.61	0.43
Area, sample set 3	3	—	0.39	0.30
Area, Pre-work	5	—	< 0.005	< 0.005

items in an attic with Zonolite located in the perimeter wall cavities. These data indicate an average exposure of 0.12 s/cc during cleaning, a value 60 times higher than the background measurements collected in the same area before the cleaning activity.

Cleaning of Storage Area in an Attic Fully Insulated with Zonolite

Five area air samples were collected before the start of cleaning activities. No asbestos structures were detected in these samples. A concentration of < 0.005 s/cc (limit of detection) was reported. During the cleaning activity the personal exposure measurements for the worker measured by PCM ranged between 2.71 and 3.00 f/cc with a TWA during the 18-minute time period of 2.87 f/cc. During the 18-minute time period the personal exposure measurements for the assistant measured by PCM ranged between < 0.55 and 1.05 f/cc, with a TWA of 0.65 f/cc.

Three sets of three area air samples (nine total) were collected during the cleaning activity and analyzed by TEM. Results were reported for structures greater than 0.5 μm in length and for structures > 5 μm in length. The TWA during a 16-minute time period for the three samples in the group closest to the cleaning activity was 0.88 s/cc and 0.61 s/cc. The TWA during a 16-minute time period for the next closest set of three area air samples was 0.61 s/cc and 0.43 s/cc. The TWA during a 16-minute time period for the farthest set of three area air samples was 0.39 s/cc and 0.30 s/cc. The results for the air samples collected for this cleaning activity are summarized in Table 2.

Three surface dust samples collected from the wood boards before cleaning contained 99,200 s/cm^2, 34,200 s/cm^2, and 96,600 s/cm^2. One sample of dust and other fine particles beneath spilled ZAI from a wooden surface contained 1.9 million s/cm^2.

From these data it may be concluded that persons cleaning an attic directly impacting Zonolite insulation will be exposed to significant concentrations of amphibole asbestos. The worker exposure was measured at almost 1000 times the background samples collected before the cleaning activity.

Cutting a Hole in the Ceiling of a Living Space Below Zonolite Attic Insulation

Prior to cutting the hole in the ceiling a set of three area air samples were collected in a second-floor bedroom. The TEM analysis found an average of 0.023 s/cc and 0.017 s/cc for structures > 5 μm in length. During the cutting process the worker and the assistant each wore two air sampling pumps for samples to be analyzed by PCM and TEM. Due to the dusty nature of the work, four sequential samples were taken for each pump (16 total). Four sequential samples were also collected at each of three area air sampling locations. These area samples were all analyzed by TEM.

The four PCM samples collected on the worker ranged from 1.42 f/cc to 14 f/cc, with a TWA of 5.8 f/cc during the 26-minute period. The four PCM samples collected on the assistant ranged from 0.81 f/cc to 16 f/cc, with a TWA of 5.4 f/cc during the 28-minute period. The difference between the 26 minute sample set and the 28 minute sample set is due the time needed to change filter cassettes on the sampling pumps.

The four TEM samples collected on the worker ranged from "not detected" (< 0.43 s/cc) to 4.98 s/cc (2.85 s/cc > 5 μm). The 26-minute TWA for the worker was 2.48 s/cc (1.32 s/cc > 5 μm). The four TEM samples collected on the assistant ranged from "not detected" to 1.83 s/cc (all structures were > 5 μm). The 28-minute TWA for the assistant was 0.80 s/cc (> 5 μm).

The three sets of four TEM area air samples collected in the same room had TWA values of 0.51 s/cc (set 1), 0.57 s/cc (set 2), and 0.77 s/cc (set 3). Considering only structures > 5 μm, the corresponding values were 0.41 s/cc (set 1), 0.54 s/cc (set 2), and 0.60 s/cc (set 3).

The data demonstrated that peak exposures occurred during the last five minutes of cutting the hole, when approximately 0.8 ft^3 of Zonolite spilled from the ceiling to the floor, a distance of about 9′. The TEM personal samples found 4.98 s/cc (2.85 s/cc > 5 μm) for the worker and 1.83 s/cc (all > 5 μm) during this phase of the work. The area air samples were similarly elevated during this phase of the work. The air sampling data are summarized in Table 3.

Three bulk samples of ZAI were collected and each found to contain less than 1% amphibole asbestos by PLM. A bulk sample of the ceiling that was cut was also analyzed by PLM for asbestos. The ceiling consisted of wood lathe, hard plaster, finish plaster, 1⁄4″ gypsum wallboard with wallpaper, and a stippled finish coat. Approximately 7% chrysotile asbestos was found in the stippled finish coat. No asbestos was found in the other materials. Accordingly, the ceiling system material cut was less than 1% chrysotile. Only Libby amphiboles were detected in the air samples.

Cutting a plaster/wallboard/wood ceiling is a dusty operation. The PCM method of measuring fiber concentrations in such an atmosphere is not a good pre-

TABLE 3 Summary of Air Sampling Results While Cutting Hole in Ceiling Below Attic with Zonolite Insulation

Sample Location	Number of Samples n	PCM TWA f/cc	TEM TWA s/cc	s/cc > 5 µm	PCME (f/cc)
Worker, personal	4,4	5.8	2.48	1.32	1.16
Assistant, personal	4,4	5.4	0.80	0.80	0.50
Area, sample set 1	4	—	0.51	0.41	0.38
Area, sample set 2	4	—	0.57	0.54	0.54
Area, sample set 3	4	—	0.77	0.60	0.56
Area, before activity	3	—	0.023	0.017	0.013

dictor of asbestos exposure. The TEM data provides the best exposure information in this instance since the method can distinguish between asbestos and non-asbestos structures. The use of the direct TEM method to measure asbestos in an atmosphere with considerable non-asbestos dust is a concern.

From this data it may be concluded that persons cutting a hole into a ceiling below Zonolite insulation will be exposed to significant concentrations of amphibole asbestos. The worker exposure was over 100 times the concentration in the background samples collected before the activity.

Moving Aside Zonolite Attic Insulation Using the W. R. Grace & Co. Method[10]

Before moving any ZAI three area air samples were collected for TEM analyses. No asbestos structures were detected in these samples. A detection limit of less than 0.002 s/cc is reported.

Personal samples were collected on the worker and the assistant during the activity. Four sequential samples were collected to prevent overloading of the filters for each sample set. Three sets of four area samples (12 total) were collected during this activity. The worker exposure was measured by four PCM samples and four TEM samples. For the assistant, both the PCM and TEM analyses were performed on the PCM filters only since the TEM filters were voided due to a sampling malfunction (crimped sampling tube).

The PCM results for the worker ranged from 4.61 f/cc to 16.24 f/cc, with a 34-minute TWA of 12.5 f/cc. The PCM results for the assistant ranged from 2.29 f/cc to 4.25 f/cc, with a 34-minute TWA of 3.12 f/cc. The TEM results for the worker ranged from 1.01 s/cc to 10.6 s/cc (1.01 s/cc to 8.58 s/cc > 5 µm), with a 34-minute TWA of 6.29 s/cc (4.85 s/cc > 5 µm). The TEM results for the assistant ranged from 4.35 s/cc to 6.42 s/cc (1.16 s/cc to 4.67 s/cc > 5 µm), with a 34-minute TWA of 5.50 s/cc (2.74 s/cc > 5 µm).

The TEM results for the three sets of area air samples as 34-minute TWAs were 3.78 s/cc (set 1), 1.86 s/cc (set 2), and 1.25 s/cc (set 3). Considering only structures greater than 5 µm, the 34-minute TWAs were 3.17 s/cc (set 1), 1.48 s/cc (set 2), and 0.90 s/cc (set 3). The results for all the area and personal samples are summarized in Table 4.

Moving Aside Zonolite Attic Insulation Using the Homeowner Method

A set of three background samples were collected from the attic before starting the activity. No asbestos structures were detected on these samples, and an average of < 0.003 s/cc was reported. The same sampling protocol was followed as was performed when moving the Zonolite using the Grace method.

The PCM results for the worker ranged from 9.48 f/cc to 18.81 f/cc, with a 31-minute TWA of 14.4 f/cc. The PCM results for the assistant ranged from 0.64 f/cc to 10.4 f/cc, with a 32-minute TWA of 4.98 f/cc. The TEM results for the worker ranged from 11.8 s/cc to 15.0 s/cc (8.4 s/cc to 12.1 s/cc > 5 µm), with a 31-minute TWA of 13.0 s/cc (10.3 s/cc > 5 µm). The TEM results for the assistant ranged from < 0.35 s/cc to 4.23 s/cc (< 0.35 to 3.82s/cc > 5 µm), with a 32-minute TWA of 2.38 s/cc (1.89 s/cc > 5 µm).

TABLE 4 Summary of Air Sampling Results During Moving Zonolite Attic Insulation Using the W.R. Grace Method

Sample Location	Number of Samples n	PCM TWA f/cc	TEM TWA s/cc	s/cc > 5 µm	PCME (f/cc)
Worker, personal	4,4	12.5	6.29	4.85	4.48
Assistant, personal	4	3.12	5.50	2.74	2.74
Area, sample set 1	4	—	3.78	3.17	2.90
Area, sample set 2	4	—	1.86	1.48	1.40
Area, sample set 3	4	—	1.25	0.90	0.82
Area, before activity	3	—	< 0.002	< 0.002	< 0.002

TABLE 5 Summary of Air Sampling Results During Moving Zonolite Attic Insulation Using the Homeowner Method

Sample Location	Number of Samples n	PCM TWA f/cc	TEM TWA s/cc	s/cc >5 µm	PCME (f/cc)
Worker, personal	4,4	14.4	13.0	10.3	9.27
Assistant, personal	4	4.98	2.38	1.89	1.75
Area, sample set 1	4	—	1.21	1.07	0.90
Area, sample set 2	4	—	2.00	1.57	1.47
Area, sample set 3	4	—	3.04	2.38	2.26
Area, before activity	3	—	< 0.003	< 0.003	< 0.003

The TEM results for the three sets of area air samples as TWAs were 1.21 s/cc (set 1, 28 minutes), 2.00 s/cc (set 2, 39 minutes), and 3.04 s/cc (set 3, 39 minutes). Considering only structures greater than 5 µm, the TWAs were 1.07 s/cc (set 1), 1.57 s/cc (set 2), and 2.38 s/cc (set 3). The results for the air samples are summarized in Table 5.

The results of sampling during the two methods of moving aside ZAI demonstrated that neither method effectively controls the generation of amphibole asbestos dust. Evaluation of the Grace method found the worker exposure to be 3100 times the levels in the background measurements, and analytical results of the homeowner method indicated the worker exposure to be 4300 times the levels in the background measurements. A review of the workers' individual sample results showed a significant exposure reduction during the last nine minutes of the task using the Grace method. This was likely due to the use of the HEPA-filtered vacuum to remove dust from between the attic floor joists during this time frame. Personal sampling results indicated 18.81 f/cc without the HEPA vacuum and 4.61 f/cc with the HEPA vacuum. A similar reduction was seen in the TEM data. Visually, the air in the vicinity of the HEPA vacuum (and the worker) became clearer. It appears the HEPA vacuum was functioning not only to scrub dust particles from the air, but also to capture dust at the surface.

Both methods of moving ZAI were dusty procedures. However, since much of the airborne fibrous dust was amphibole asbestos, the limitations of using PCM and direct TEM were not as pronounced. In a different attic that might contain ZAI and another product, such as treated cellulose or mineral wool, interference from non-asbestos fibers would likely make sampling and analysis more challenging since the non-asbestos fibers would be interpreted as asbestos by the PCM method. The TEM method can disregard the non-asbestos fibers, but in a dusty environment may make the analysis difficult, if not impossible. In some instances it may be necessary to use the indirect TEM preparation technique to overcome the overloaded sample.

The use of water to mist the ZAI was not very effective as a dust suppressant. This may have been due to the thickness of the attic insulation and the micaceous product itself. Caution should be used when using water on Zonolite attic insulation. Old and poorly insulated electric wiring is often found in the loose attic fill material. This poses an electric shock hazard.

Removal of Zonolite Attic Insulation with a Shop Vacuum from the Top of Perimeter Wall Cavities

Before beginning the removal of ZAI from the top of perimeter wall cavities, a set of four area air samples were collected to establish the background concentration of asbestos. No asbestos was detected in these samples and the limit of detection values of less than 0.0016 s/cc were reported.

Personal samples were collected on the worker and the assistant during the activity. Four sequential samples were collected to prevent overloading of the filters for each sample set. Four sets of four area samples (16 total) were collected during this activity. The worker's exposure was measured by four PCM samples and four

TABLE 6 Summary of Air Sampling Results During Removal of Zonolite Insulation with a Shop Vacuum from the Top of Wall Cavities

Sample Location	Number of Samples n	PCM TWA f/cc	TEM TWA s/cc	s/cc >5 µm	PCME (f/cc)
Worker, personal	4,4	2.90	1.47	0.98	0.97
Assistant, personal	4	2.90	1.69	1.10	1.03
Area, sample set 1	4	—	0.52	0.37	0.32
Area, sample set 2	4	—	0.67	0.45	0.40
Area, sample set 3	4	—	0.89	0.57	0.47
Area, sample set 4	4	—	1.00	0.73	0.63
Area, before activity	4	—	< 0.0016	< 0.0016	< 0.0016

TABLE 7 Summary of Air Sampling Results

Activity Evaluated	Personal Samples			Area Samples	
	f/cc	s/cc	s/cc >5µm	s/cc	s/cc >5 µm
Cleaning items in an attic	1.54	< 0.42	< 0.42	0.08	0.07
Cleaning storage area in an attic	2.87	4.00	2.58	0.63	0.47
Cutting hole in ceiling below ZAI	5.80	2.48	1.32	0.62	0.52
Moving ZAI-manufacturer method	12.5	6.29	4.85	2.30	1.85
Moving ZAI-homeowner method	14.4	13.00	10.30	1.82	1.47
Shop vacuum removal	2.90	1.47	0.98	0.77	0.53
No activity	—	—	—	< 0.003	< 0.003

TEM samples. For the assistant, eight samples were also collected, but the PCM and TEM analyses were performed on the PCM filters (0.8 µm pore size) since the TEM samples were voided due to sampling malfunction (crimped sampling tube).

The PCM results for the worker ranged from 1.19 f/cc to 5.28 f/cc, with a 46-minute TWA of 2.90 f/cc. The PCM results for the assistant ranged from 1.47 f/cc to 4.81 f/cc, with a 46-minute TWA of 2.90 f/cc. The TEM results for the worker ranged from 1.05 s/cc to 2.16 s/cc (0.58 s/cc to 1.32 s/cc, >5 µm), with a 46-minute TWA of 1.47 s/cc (0.98 s/cc, > 5 µm). The TEM results for the assistant ranged from 0.67 s/cc to 2.15 s/cc (<0.67 s/cc to 1.79 s/cc, > 5 µm), with a 46-minute TWA of 1.69 s/cc (1.10 s/cc, > 5 µm).

The TEM results for the four sets of area air samples as TWAs were 0.52 s/cc (set 1, 43 minutes), 0.67 s/cc (set 2, 42 minutes), 0.89 s/cc (set 3, 42 minutes), and 1.00 s/cc (set 4, 45 minutes). Including only structures greater than 5 µm, the TWAs were 0.37 s/cc (set 1), 0.45 s/cc (set 2), 0.57 s/cc (set 3), and 0.73 s/cc (set 4). The results for the air samples are summarized in Table 6.

Just prior to the removal activity, four sheets of aluminum foil were placed on surfaces to collect dust which might settle during the activity and for a period of 20 to 33 minutes following completion of the activity. The total collection time was 65 to 78 minutes. No asbestos structures were found in two of the samples (< 300 s/cc reported as the limit of detection). The other two samples found 300 s/cm^2 and 700 s/cm^2 of amphibole asbestos. The data, when viewed together with the area air sampling, indicate that one hour may not be sufficient time to allow for the asbestos structures to settle out of the air.

The worker and the assistant exposure data were very similar for this activity. The likely cause was that the worker and assistant worked together to dump the Zonolite from the vacuum into plastic bags. This was a visually dusty operation.

The data from the use of a standard shop vacuum to remove Zonolite insulation demonstrated that this activity resulted in significant exposure to amphibole asbestos. The worker exposure for this study was found to be 735 times the levels measured in the background samples collected before the activity began.

Additional Observations

All air sampling results from our studies are summarized in Table 7. These studies were limited to only three homes with ZAI. Under contract to the US EPA, Versar, Inc. has also conducted a series of studies to characterize exposures from vermiculite attic insulation.[18] Some of these studies consisted of activities in a small containment, a large containment, and one home in Vermont. The activities they considered were as follows:

1. installing and removing vermiculite attic insulation;
2. performing wiring/small renovations in an attic with vermiculite;
3. using an attic with vermiculite insulation as storage space;
4. living in a house where disturbances to vermiculite insulation occurs; and
5. measuring background levels in a house with vermiculite attic insulation.

Versar conducted air sampling before, during, and after 20 activities. In general, they found significantly increased airborne concentrations when the vermiculite attic insulation was directly disturbed.

Additional studies in other homes evaluating exposures from these types of activities as well as other activities may be helpful. While Versar's studies addressed measured amphibole from asbestos-contaminated vermiculite attic insulation, vermiculite was also commonly used as fill-in for concrete block walls. The authors of this present study are not aware of published studies evaluating exposures from vermiculite filled block walls. This is an area deserving future research.

The EPA has conducted several studies evaluating exposures to ZAI. These studies as well as guidance for homeowners may be found at http://www.epa.gov/asbestos/pubs/verm.html. In the US and Canada ZAI was used in homes, with much of the insulation coming from the Libby, MT deposit. To what extent this same material may have been exported outside of these two countries is unknown.

Analyses conducted in the field and on laboratory blank samples indicated there was no systematic con-

tamination of the samples in the field or the laboratory. Samples collected outdoors failed to detect any amphibole asbestos.

The background samples collected in the attics of the three houses indicated that absent any disturbance, there was not an elevated concentration of asbestos in the air. Similar sampling should be conducted in homes during high wind storms. Anecdotal information from at least one homeowner indicates that some Zonolite insulation is blown out from wall cavities under certain circumstances.

Home C had an attic fan that may have been responsible for the displacement of some of the ZAI. Another interesting investigation would be to determine the exposures among occupants in homes with ZAI when attic fans are operating.

CONCLUSIONS

This series of studies indicatesd that ZAI present in the attic of homes, if undisturbed, seems not to result in elevated exposures. Likewise, the data presented here demonstrated that many routine cleaning, maintenance, and remodeling activities that disturb ZAI can generate significant airborne amphibole asbestos exposures. A review of Tables 2 to 6 demonstrates that the OSHA excursion limit for asbestos of 1 f/cc during any 30-minute period was often exceeded. Depending on the length of the work, the OSHA eight-hour permissible exposure limit (PEL) would often have been exceeded. When such work in attics are performed by homeowners, the OSHA regulations do not apply. This is one of the gaps in regulatory coverage for asbestos.

There is a need to assess what exposures occur during the demolition of homes with ZAI and evaluate control measures that will eliminate or minimize the exposures experienced by workers and the community. A standard protocol for the removal of ZAI from homes should be developed.

Analyses conducted of the bulk ZAI in these homes and other buildings generally results in amphibole asbestos concentrations of less than 1% and often less than 0.1 %. However, the exposure data presented here, and the exposure data from the Manitoba building referenced earlier, demonstrate that significant exposures can still occur. These exposures can be in excess of current regulatory exposure limits.

To what extent these results may be generalized to the disturbance of other materials in buildings with less than 1% asbestos, such as some wall plasters, has not been established. However, it would be prudent to evaluate exposures for materials where asbestos is detected in the bulk samples at any level. One type of Zonolite vermiculite was also used in some fireproofing for structural steel with no added asbestos. We are not aware of any published data evaluating exposures during disturbances of this material. Publication of such information could assist building owners and managers in reducing future exposures.

Requiring the control of exposures arising from building materials containing less than 1% asbestos has a number of policy implications. Traditionally the regulatory agencies, such as OSHA and EPA, have set a limit of 1% to trigger the identification of a material as "asbestos-containing." With improved analytical techniques, regulatory agencies should revisit the definition of an asbestos-containing material to include some at levels below 1%.

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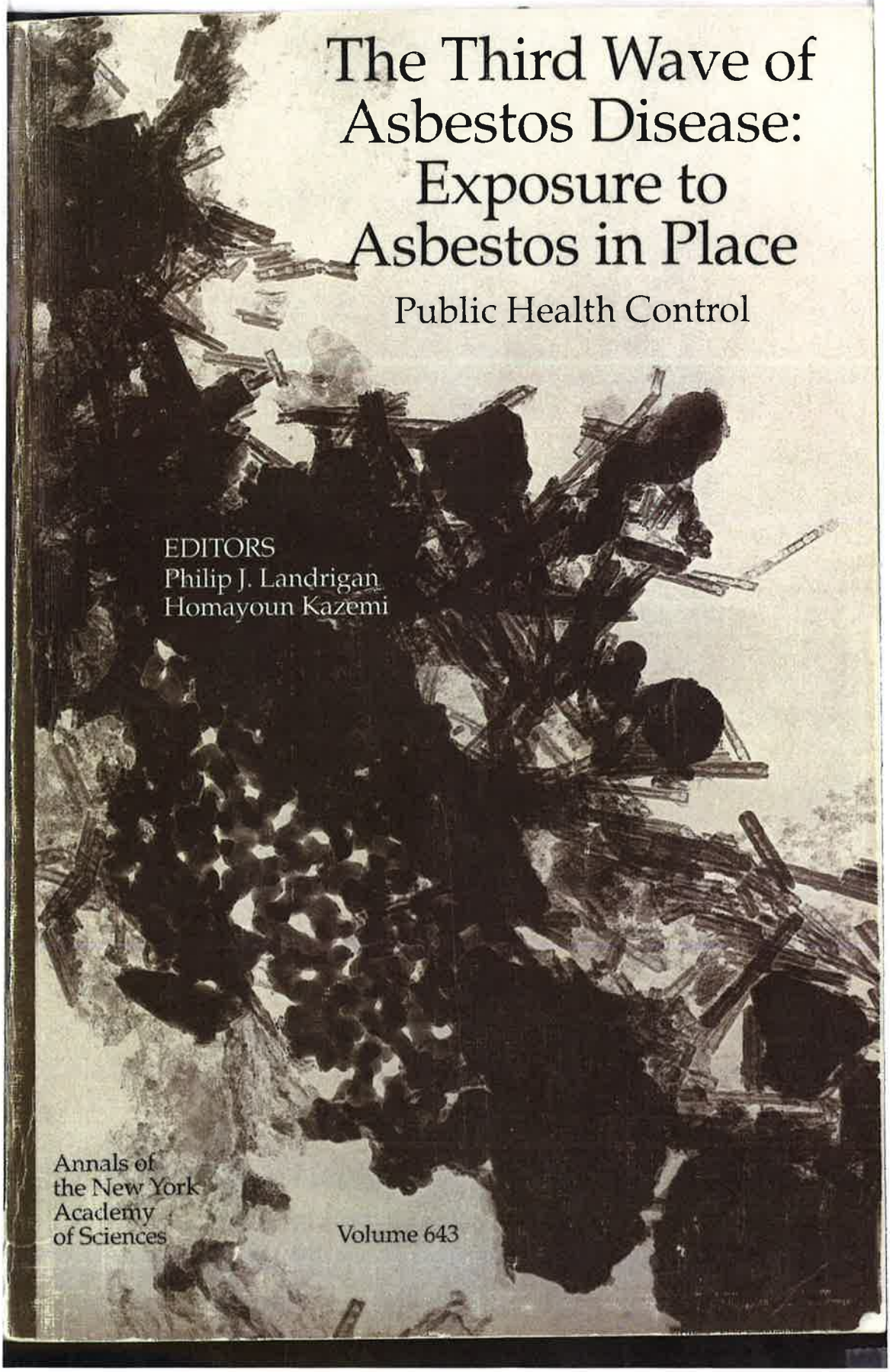
Exhibit 17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Cover: *The cover of the paperbound edition of this book shows a photomicrograph of chrysotile fibrils in mesotheliomatous tissue in a human. Photograph courtesy of Dr. Y. Suzuki.*

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THE THIRD WAVE OF ASBESTOS DISEASE: EXPOSURE TO ASBESTOS IN PLACE[a]

PUBLIC HEALTH CONTROL

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[a] This volume is the result of a conference entitled **The Third Wave of Asbestos Disease: Exposure to Asbestos in Place—Public Health Control** held by the Collegium Ramazzini on June 7–9, 1990 in New York, New York.

Part 10. Populations at Risk: The Construction Industry

Part 11. Other Populations at Risk

Part 12. Asbestos Exposure of Nonoccupational Groups

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THE THIRD WAVE OF ASBESTOS DISEASE: EXPOSURE TO ASBESTOS IN PLACE

PUBLIC HEALTH CONTROL

NORTON NELSON
1910–1990

In Memoriam: Norton Nelson

It is fitting that this conference be dedicated to the memory of Dr. Norton Nelson. Although Dr. Nelson was not himself a physician, he was dedicated to the prevention of occupational and environmental diseases. As a member of the Collegium Ramazzini and the recipient of the Ramazzini Award in 1985, Dr. Nelson exemplified in his own life the ideals and philosophy espoused centuries ago by Bernardino Ramazzini. Were he alive today, Dr. Nelson would be with us in body as he very much is in spirit.

Occupationally and environmentally induced respiratory diseases were a special interest of Dr. Nelson's, and an area to which he made many important contributions in the course of his productive life.

In addition to his many scientific accomplishments, for which he will long be remembered, the world will always be especially grateful to Dr. Nelson for his vision and leadership in the creation of local, national, and international institutions for the advancement of occupational and environmental health.

As was so ably stated by Dr. David Rall, Director of the National Institute of Environmental Health Sciences, "Dr. Nelson can rightly be considered the father of the second generation of environmental public health, the first being focused on vector-borne illnesses, pure water supplies, sanitation, and food safety. This second generation is directed towards the possible health effects humans create in an industrialized society. Furthermore, through his tutelage and example, a third generation of environmental scientists is emerging. These scientists will understand both what the threats and dangers are and how they act, and will utilize and apply techniques such as molecular biology and computer modelling to develop approaches to prevention and mitigation."

We are all richer for the legacy that Dr. Nelson has left us. At the same time, we share with his wife Rose, who is with us, a profound sadness in the loss of our beloved friend.

ARTHUR C. UPTON

Preface

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For close to sixty years data on the health hazards of asbestos have been documented in the scientific literature. Twenty-six years ago, in 1965, the New York Academy of Sciences published the seminal document *Biological Effects of Asbestos*,[1] which conveyed this knowledge to the general public. That publication was followed twelve years later by the release of the report entitled *Health Hazards of Asbestos Exposure*.[2] Those two reports documented the etiologic associations between asbestos exposure and the development of asbestosis, lung cancer, and mesothelioma. They detailed the extent of the asbestos-related disease epidemic in the United States, and they provided the essential basis for prevention. Now, with the publication of this report, *The Third Wave of Asbestos Disease: Exposure to Asbestos in Place—Public Health Control,* the latest developments in the continuing asbestos experience are documented.

The need to convene yet another conference on asbestos disease is sad testimony to how much has yet to be accomplished in the dissemination of information to protect public health. On a positive note, the interest generated by the convening of this meeting under the auspices of the Collegium Ramazzini is proof that concern about the health effects of occupational and environmental exposures remains high.

The first phase of asbestos disease was associated with work in the mining and milling of ore and the manufacture of asbestos products. It began to be recognized in the 1920s and its legacy is still with us.

The second phase came to be recognized in persons who used asbestos products. The numbers of persons so exposed was very large; conditions of exposure were more complex, and not readily amenable to control. In a world habituated to acute illness and injury, the concept of latency in the development of chronic or late disease was not appreciated. Persons exposed in the 1940s and 1950s are only now reaching their sixth and seventh decades, and their risk of illness induced by asbestos is very great indeed.

Although the documentation of asbestos hazards has resulted in some mandated reduction of grosser exposures in some settings, it has also led to the recognition that even very short-term and/or low-level exposures pose risk. And this risk now extends to other settings, other populations.

Asbestosis, lung cancer, mesothelioma, and a wide range of other types of cancer have been shown to be caused by exposure to asbestos. Epidemiologic studies conducted worldwide have established that *all* forms of asbestos have the capacity to cause these illnesses. These studies have shown that the incidence of asbestosis, lung cancer, and other tumors is quantitatively related to cumulative asbestos exposure in a positive dose–response relationship. By the end of the century, an estimated 30,000 to 50,000 premature deaths will have occurred in New York State alone as the result of occupational exposures to asbestos that occurred prior to 1980. Today, as buildings constructed with asbestos over the past six decades begin to age and deteriorate, serious potential exists for a third phase of asbestos disease among persons engaged in the repair, renovation and

demolition of these buildings. Potential also exists for serious environmental exposure to asbestos among residents, tenants and users of these buildings, such as school children, office workers, maintenance workers, and the general public. The Centers for Disease Control, the American Academy of Pediatrics, and the U.S. Environmental Protection Agency have projected that over the next 30 years approximately 1,000 cases of mesothelioma and lung cancer will occur among persons in the United States exposed to asbestos in school buildings as school children.

The conference held in New York on June 6, 7, and 8, 1990 under the aegis of the Collegium Ramazzini, the proceedings of which constitute this volume, was called in an effort to understand better the kinds of problems we face and to address better the issues of low-level exposure and what its consequences might be. Scientific information, carefully analyzed and reviewed, offers the best chance of helping us decide how to meet this next challenge from a public health point of view. Change is often perceived as a threat, and there will always be those who opt for the status quo. Yet, if we do not move forward, we shall be retreating by allowing the continued exposure to hazards for many less empowered persons, all over the world, hazards that we would not readily assume for ourselves and our children.

The presenters and discussants at this conference represent a multidisciplinary, international group of researchers with experience in the evaluation of these newly recognized "at risk" populations. Their contributions will do much to address the adage that "science is necessary but not sufficient." The contributions to accuracy in matters scientific and editorial, by Dr. Martha Kimball and Marise Burger are gratefully acknowledged, as is the editorial guidance of Bill Boland, Justine Cullinan, and Sheila Kane.

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Mineral Fiber Content of Lung Tissue in Patients with Environmental Exposures: Household Contacts vs. Building Occupants

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In recent years, there has been considerable debate within the scientific community regarding the potential risks due to exposure to asbestos in nonoccupational settings.[1–6] Of particular concern is the possible risk for development of malignant mesothelioma, which is known to occur in some cases after brief or low-level exposures to asbestos.[7–10] However, only a few reports have been published concerning the determination of mineral fiber burdens within the lungs of persons with environmental (i.e., nonoccupational) exposure to asbestos.[11–13] One of us (V.L.R.) has had the opportunity to examine the mineral fiber content of lung tissue in ten cases where the only known exposure to asbestos was as a household contact of an asbestos worker or as an occupant in a building containing asbestos insulation. It is the purpose of the present report to describe the fiber burdens in these ten cases and to compare these results with the findings in various categories of occupational exposure to asbestos.

MATERIALS AND METHODS

Case Selection. Included in this report are all cases from my consultation files for which the only known exposure to asbestos was as a household contact of an asbestos worker (six cases) or as an occupant of a building containing asbestos materials (four cases), and for which lung tissue (formalin-fixed or paraffin-embedded) was available for measurement of mineral fiber content. The demographic and pathologic findings, exposure history, and asbestos body and mineral fiber concentrations in these ten cases are summarized in TABLES 1 and 2. For the six patients with malignant (diffuse) mesothelioma, previously published histologic criteria were used to establish the diagnosis on tissues obtained either at autopsy (two cases) or surgical resection (two cases) or both (two cases).[10]

Mineral Fiber Analysis. Tissue mineral fiber content was determined using the sodium hypochlorite digestion procedure, the details of which have been reported previously.[14,15] In brief, formalin-fixed lung parenchyma with a wet weight between 0.25 and 0.35 gm was minced with a clean scalpel blade and digested in 5.25% sodium hypochlorite solution (commercial bleach) with constant gentle

TABLE 1. Demographic, Pathologic, and Exposure Information and Asbestos Content of Lung in Six Household Contacts of Asbestos Workers

Case No.	Age (yr)/ Sex	Exposure	Diagnosis	AB/gm (LM)	UF/gm (SEM)
1	62/F	Wife of shipyard insulator with asbestosis; 29 yr	Pleural mesothelioma	8,200	ND
2	33/F	Daughter of insulator with asbestosis; 25 yr	Pleural mesothelioma	2,330	17,000
3	63/F	Wife of insulator with asbestosis and lung cancer; yrs	Small cell/large cell carcinoma of lung; mild asbestosis	3,670	120,000
4	59/F	Wife of insulator with asbestosis and lung cancer; 23 yrs.	Small cell carcinoma of lung; PPP	1,060	57,000
5	73/F	Wife of insulator with lung cancer and asbestosis; yrs	Bronchioloalveolar cell carcinoma of LUL	400	23,700
6	57/F	Wife of shipyard worker; 1–2 yr	Pleural mesothelioma	2	24,300

ABBREVIATIONS: AB/gm (LM) = asbestos bodies per gram of wet lung as determined by light microscopy; UF/gm (SEM) = uncoated fibers 5μ or greater in length per gram of wet lung as determined by scanning electron microscopy; PPP = parietal pleural plaques; LUL = left upper lobe; ND = not done.

agitation. The residue was collected on 0.4 μ-pore-sized polycarbonate filters, one of which was mounted on a glass slide for asbestos body quantification by light microscopy (LM) at 200× magnification. The other was mounted on a carbon disc with colloidal graphite, sputter-coated with gold, and examined by scanning electron microscopy (SEM) at a screen magnification of 1000×.[16] Fibers were defined

TABLE 2. Demographic, Pathologic, and Exposure Information and Asbestos Content of Lung in Four Occupants of Buildings with Asbestos-Containing Materials

Case No.	Age (yr)/ Sex	Exposure	Diagnosis	AB/gm (LM)	UF/gm (SEM)
7	46/M	Worked in building with ACM; 20 yr	Adenocarcinoma of lung	14	25,000
8	58/F	Teacher in building with ACM; 18 yr	Pleural mesothelioma; PPP	2.8	13,000
9	45/M	Attended school containing asbestos; 12 yr	Peritoneal mesothelioma	1.0	6120
10	53/M	Accountant in building with ACM; 18 yr	Pleural mesothelioma	<0.2	6370

ABBREVIATIONS: AB/gm (LM) = asbestos bodies per gram of wet lung as determined by light microscopy; UF/gm (SEM) = uncoated fibers 5μ or greater in length per gram of wet lung as determined by scanning electron microscopy; PPP = parietal pleural plaques; ACM = asbestos-containing materials.

as particles with an aspect ratio (length : diameter) of at least 3 : 1 and roughly parallel sides, and particles meeting these criteria and with a length of 5 μm or greater were counted. From these data, fiber density on the filter surface and numbers of fibers per filter could be determined. Asbestos bodies and uncoated fibers were enumerated separately, and results reported as asbestos bodies or uncoated fibers 5 μm or greater in length per gram of wet lung tissue.[16] In two cases (Cases 9 and 10, TABLE 2), an additional 5-gram sample of lung tissue was processed for asbestos body quantification using the technique of Smith and Naylor.[17]

In three cases (Cases 1, 3 and 4, TABLE 1), only paraffin blocks of lung parenchyma were available for analysis. In these cases, tissue was recovered from the block, deparaffinized in xylene, and rehydrated to 95% ethanol as previously described.[15,18] Digestion was then performed as described above. The filter was cut in half with a scalpel blade, and one half was mounted on a glass slide for asbestos body quantification by LM, whereas the other half was mounted on a carbon disc and examined by SEM. The results were multiplied by a correction factor (0.7), which takes into account the difference in weight between formalin-fixed lung and lung that has been processed into paraffin.[15]

The chemical composition of mineral fibers was determined by means of energy-dispersive spectrometry in nine of the ten cases. Five to thirty consecutive fibers were analyzed per case and classified as asbestiform (amosite, crocidolite, tremolite, anthophyllite, actinolite, or chrysotile) or nonasbestiform on the basis of morphology and chemical composition as previously described.[15,16]

Additional studies were performed in one case (Case 8, TABLE 2) to further characterize the mineral content of lung tissue. Paraffin-embedded lung parenchyma was deparaffinized in xylene (three changes, 2 hours each) and ashed in a low-temperature plasma asher for 100 hours. The dry weight of four combined specimens in this case was 0.18 gram. After ashing was complete, the remaining residue was suspended in 24 ml of filtered, deionized water and then sonicated for 10 minutes. The suspension was then filtered through a 0.45 μ-pore-sized mixed cellulose ester filter, which was then prepared by the direct method for examination by transmission electron microscopy, selected area electron diffraction, and energy-dispersive spectrometry (TEM/SAED/EDS).[19] Also examined with the same method was tissue obtained from five patients who had died approximately at the same time and in the same institution as Case 8. These patients had died from coronary artery disease (two cases), pulmonary embolism, carcinoma of the colon, or cirrhosis (one each). Reagent blanks were also prepared as described above, but with tissue omitted.

In addition, a plaster sample was obtained from the high school where case 8 was employed and was analyzed for its mineral content by means of polarized light microscopy with dispersion staining[20] and by TEM/SAED/EDS. Also, the weight percent soluble component was determined by dissolution in a mild hydrochloric acid solution.

RESULTS

The tissue asbestos content of the six household contacts of asbestos workers is summarized in TABLE 1. All were women with ages ranging from 33 to 73. Three of these patients had pleural mesothelioma and three had lung cancer. One of the latter also had mild asbestosis and one had parietal pleural plaques. Case 5 was a nonsmoker. The husband in four cases and the father in one case had worked as

asbestos insulators. Each had been diagnosed as having asbestosis and three also had lung cancer. The asbestos body (AB) counts among the six household contacts ranged from 2 to 8,200 AB/gm, with a median value of 1,700 AB/gm. The contents of uncoated fibers (UF) 5 microns or greater in length ranged from 17,000 to 120,000 UF/gm, with a median count of 24,300 UF/gm. In comparison, our normal range for asbestos bodies as determined in 84 cases with no evidence of asbestos exposure or an asbestos-related disease is 0–20 AB/gm.[15,16,18] The median uncoated fiber count for 20 patients with macroscopically normal lungs at autopsy and no history of asbestos exposure was 3,100 UF/gm[16] (and unpublished observations).

The tissue asbestos content of the four building occupants is summarized in TABLE 2. There were three men and one woman, with ages ranging from 45 to 58. Two of these had pleural mesothelioma, one had peritoneal mesothelioma, and one had adenocarcinoma of the lung. The latter was a nonsmoker. All four had either worked or attended school in buildings with asbestos-containing materials for periods ranging from 12 to 20 years. The asbestos body counts among the four building occupants ranged from less than 0.2 to 14 AB/gm, with a median value of

TABLE 3. Asbestos Content of Lung Tissue by Exposure Category[a]

	n	AB/gm (LM)	UF/gm (SEM)
Insulation workers	59	20,400	224,000
Shipyard workers (other than insulators)	60	3,600	37,000
Other asbestos workers	24	2,360	68,800
Household contacts	6	1,700	24,300
Railroad workers	10	55	28,800
Brakeline work or repair	8	50	15,400
Manual laborer	15	20	8,830
Other	18	2.9	2,910
Building occupants with ACM	4	1.9	9,680

[a] Data are presented as median values. For other abbreviations, see footnotes to TABLES 1 and 2.

1.9 AB/gm. All are within our normal range of 0–20 AB/gm. The content of uncoated fibers 5 microns or greater in length ranged from 6,120 to 25,000 UF/gm, with a median count of 9680 UF/gm. The latter exceeds the median count of 3,100 UF/gm found in our 20 patients with macroscopically normal lungs and no known exposure to asbestos.

TABLE 3 compares the tissue asbestos content in these 10 cases with environmental exposure with that of 161 occupationally exposed individuals and 33 with no known occupational exposure. It can be seen that in terms of asbestos body concentrations, household contacts rank fourth and have levels that are comparable to those of shipyard workers other than insulation workers and other asbestos workers (including asbestos cement workers, asbestos textile workers, chemical maintenance workers, welders, machinists, filter manufacturers, roofing plant workers, refinery workers, sheet-metal workers, and industrial workers with exposure to asbestos not further specified). Building occupants rank last with regard to asbestos body concentrations, and generally these values are the same as those in individuals with no known exposure to asbestos (including textile workers, farmers, military personnel, chemical workers, factory workers, dieticians,

TABLE 4. Energy-Dispersive Spectrometry of Fibers in Patients with Environmental Exposures

	n	Commercial Amphiboles[a]	Noncommercial Amphiboles[b]	Chrysotile	Other[c]
Household contacts	5	46 (48%)	10 (10.5%)	4 (4.2%)	35 (37%)
Building occupants	4	2 (4.4%)	9 (20%)	1 (2%)	33 (73%)

[a] Commercial amphiboles = amosite and crocidolite.
[b] Noncommercial amphiboles = tremolite, anthophyllite, and actinolite.
[c] Other = talc, silica, rutile, aluminum silicates, miscellaneous silicates, iron, and iron–chromium.

guards, musicians, salesmen, barbers, engineers, teachers, tailors, grainmill workers, building contractors, truck drivers, and office workers). Although the ranking by uncoated fiber concentration is slightly different from that for asbestos body content, the former must be considered in light of the types of fibers (asbestiform or nonasbestiform) present as determined by EDS.

The chemical composition of 95 fibers isolated from the lungs of five of the household contacts and 45 fibers isolated from the lungs of the four building occupants is summarized in TABLE 4. Almost half of the fibers from the household-contact cases were the commercial amphiboles, amosite or crocidolite, whereas fewer than 5% of the fibers from the building occupants were commercial amphiboles. On the other hand, almost three-fourths of the fibers from the building occupants were nonasbestos mineral fibers,[21,22] mostly talc, silica, rutile, and miscellaneous aluminum silicates. Noncommercial amphiboles and chrysotile accounted for a minority of fibers in both groups (15 to 22%).

Scanning electron microscopic analysis of lung tissue from Case 8 disclosed a substantial number of high aspect-ratio fibers with a chemical composition indicative of tremolite. Talc, aluminum silicate, and mica particles with a 3 : 1 or greater aspect ratio and length of 5μ or more were also identified. Further analysis of lung tissue from this case by analytical TEM confirmed the presence of talc, tremolite, chrysotile, bentonite, and perlite. These constitute five of the seven components identified in the acoustical plaster from the school where this patient was employed (TABLE 5). No more than two of these seven components were found in the lungs of the five control subjects. Additional particles found in these latter five patient's lungs included kaolinite, attapulgite, quartz, and mica.

TABLE 5. TEM/SAED/EDS Data Regarding Particulate Content of Lung in Case 8 as Compared to Five Control Subjects and Plaster from Building

	Chrysotile	Tremolite	Perlite	Talc	Bentonite	Calcite	TiO_2
Plaster	+	+	+	+	+	+	+
Case 8	+	+	+	+	+	–	–
Control A	+	–	–	+	–	–	–
Control B	–	+	–	–	–	–	–
Control C	–	–	–	–	–	–	–
Control D	–	+	–	–	–	–	–
Control E	–	–	+	+	–	–	–

NOTE: + = Present; – = not detected.

DISCUSSION

An increased risk of developing an asbestos-related disease has been reported among household contacts of asbestos workers,[2,9] presumably secondary to asbestos fibers brought home on the worker's clothing. However, there have been few reports of the analysis of pulmonary asbestos content among household contacts of asbestos workers. Whitwell *et al.*[11] described a case of mesothelioma in the son of a worker from a gas-mask factory where the workers took crocidolite home to pack into canisters. The worker's son was found to have between 50,000 and 100,000 fibers per gram of dry lung tissue as determined by phase-contrast light microscopy. (One gram of dry lung tissue is approximately equivalent to 10 grams of wet lung tissue.) Huncharek *et al.*[12] reported another case of mesothelioma in the 76-year-old wife of a shipyard machinist who dismantled boilers and other shipyard machinery for 34 years. This patient was found to have 6.5 million fibers per gram of dry lung as determined by TEM. The present study indicates that, in general, household contacts have substantially elevated pulmonary asbestos burdens, often in the range of those of individuals who are occupationally exposed to asbestos (TABLE 3). That the exposures in these women's homes were heavy is further supported by the observation that in five of the six cases, the occupationally exposed individual in the household was an insulation worker with clinically diagnosed asbestosis. Three of these individuals also had lung cancer. The median asbestos body and uncoated fiber contents of 30 insulation workers with asbestosis in the author's series are 109,000 AB/gm and 646,000 UF/gm of wet lung tissue, respectively.

There has been considerable scientific and public debate concerning possible risks of asbestos-induced disease derived from living, working, or attending school in buildings containing asbestos.[1–6] Certainly the measured air fiber levels in buildings using current methods are extremely low,[23] and no adverse health effects have been observed in at least one comparison study of workers in buildings with and without asbestos insulation.[24] However, significant levels of asbestos-contaminated dust are found in these buildings, and routine maintenance activities can disturb this dust, producing high concentrations of airborne asbestos. The present study indicates that building occupants have pulmonary asbestos burdens that are quite similar to those of individuals with no known occupational exposure to asbestos (TABLE 3), and it would be anticipated that their risks for developing an asbestos-related disease would be correspondingly low. It should be noted that exposure to asbestos as a building occupant cannot be excluded among the 18 individuals in TABLE 3 with no known occupational exposure to asbestos. However, we have no reason to believe that these individuals are anything other than representative of the background, "nonexposed" population for our area. Furthermore, not all mesotheliomas are related to asbestos exposure since spontaneous cases do occur[11] as do a few rare cases attributable to causes other than mineral fibers.[25]

There is a single case report in the literature of pleural mesothelioma developing in an individual whose only known exposure to asbestos was as an office worker in a building with asbestos-containing materials (ACM).[13] This was a 54-year-old woman who worked for many years in a building with ceiling material composed of 70% amosite asbestos. Analysis of her lung tissue demonstrated 31 million fibers per gram of dry lung by TEM, the vast majority of which were found to be amosite asbestos by EDS.[13] Our Case 8 demonstrated an unusual number of high aspect-ratio tremolite fibers within her lung parenchyma (TABLES 2 and 5). Tremolite asbestos is a recognized cause of pleural mesothelioma, accounting for

about 20% of cases according to the study by McDonald *et al.*[26] Since multiple components of the acoustical ceiling plaster from the building in which this patient worked were also found in her lung tissue samples, this is the most likely source of the tremolite asbestos fibers that were identified. There was no evidence of exposure to cosmetic talc and no evidence of household exposure on the basis of her husband's occupational history. Furthermore, the presence of histologically confirmed parietal pleural plaques is compelling evidence that this woman's pleural mesothelioma was indeed asbestos-related. Additional studies are necessary in order to determine whether such cases as these occur with sufficient frequency to be of public concern.

SUMMARY

Analysis of tissue mineral fiber content in patients with environmental exposures has seldom been reported in the past. Our studies of six household contacts of asbestos workers indicate that these individuals often have pulmonary asbestos concentrations similar to some occupationally exposed individuals. In contrast, our studies of four occupants of buildings with asbestos-containing materials indicate that these individuals often have pulmonary asbestos burdens indistinguishable from the general nonoccupationally exposed population. However, one such building occupant exposed for many years and who later developed pleural mesothelioma was studied in detail, and it was concluded that her exposure as a teacher's aide in a school building containing acoustical plaster was the likely cause of her mesothelioma.

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Exhibit 18

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Procedure for the Analysis of Talc for Asbestos

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ABSTRACT

The analysis of talc powder for asbestos is most appropriately done with a combination of polarized light microscopy (PLM), transmission electron microscopy (TEM) and in some cases a screening by X-ray diffraction (XRD). Low levels of thin asbestos fibers in talc may only be seen using the TEM analysis. Although never formally adopted by the U.S. Environmental Protection Agency (EPA), the 1993 EPA bulk method (EPA R-93) for asbestos provides the basis for the PLM portion of the method, as it is a good description of the light microscopy techniques available. The consensus method D6281 balloted and published by ASTM International provides the basis for the TEM portion of the method. The method described here has been used to investigate vintage talcum powders and talcum products currently available. Some asbestos has been found in vintage powders but with the exception of one Chinese product, asbestos was not detected in currently available powders using the talc-asbestos method described here.

Keywords: talcum, asbestos, polarized light microscopy (PLM), transmission electron microscopy (TEM), X-ray diffraction (XRD), light microscopy, National Institute for Occupational Safety and Health (NIOSH), U.S. Environmental Protection Agency (EPA), ASTM International, International Standards Organization (ISO), phase contrast microscopy (PCM), McCrone Research Institute, New York University Department of Chemistry, tremolite, chrysotile, anthophyllite, pyrophyllite, asbestiform, fibers, selected area electron diffraction (SAED), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), Asbestos Hazard Emergency Response Act (AHERA) U.S. Pharmacopeia (USP) Talc monograph, infrared spectroscopy (IR), Walter C. McCrone, Lucy McCrone

INTRODUCTION

In 1968, Cralley et al. (1), from the Occupational Health Program, National Center for Urban and Industrial Health in Cincinnati, Ohio (predecessor of the National Institute of Occupational Safety and Health — NIOSH) reported that they had examined 22 talcum products purchased off-the-shelf (representing body powder, bath powder, and all purpose powder) for fibrous and mineral content. Cralley et al. used phase contrast microscopy (PCM) and found that all of the 22 talcum products had an appreciable fiber content that ranged from 8% to 30% by count of the total talcum particulates. Although the specific fibrous materials were not identified by PCM, XRD analysis by the authors led them to believe that the fibers were predominantly fibrous talc, with the probable presence in minor amounts of other fibrous minerals, such as tremolite, anthophyllite, chrysotile and pyrophyllite. The authors remarked that the electron microscope, with its higher power of resolution, showed a number of submicron diameter particulates not visible by means of PCM, but they did not identify any of the

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fibers by electron microscopy. The authors concluded that cosmetic talcum products should be included as a source of fibers from which may be derived ferruginous bodies observed in the lungs of humans.

A number of independent scientists were involved with analyzing talcum powders in the 1970s. Walter C. McCrone Associates, Inc., in Chicago analyzed talcum powders for various groups, including NIOSH. They used PLM, XRD and TEM in their investigations. They reported finding asbestos fibers in a number of talc samples (2–5).

At the New York University Department of Chemistry one sample of talcum powder sample (referred to as #1615) was tested in 1972 (6). They reported that their initial test by XRD showed "some features in its X-ray pattern that suggested that it might contain some tremolite" and "accordingly, the specimen was subjected to a detailed microscopic examination. Both tremolite and chrysotile fibers were found to be present in the sample. It is estimated the tremolite content is about 2% by weight, and the chrysotile about 0.5%" (6).

In 1974, Rohl and Langer (7) reported on the analysis of consumer talcum powders using analytical methods for identification, characterization and quantitation of asbestos fibers that included PLM, XRD, and TEM with selected area electron diffraction, and electron microprobe techniques. They remarked that the light microscope methods had severe limitations imposed by the ultimate size resolution of the light-optical system. They reported that small particles can go unresolved and most optical properties, e.g., refractive indices, are difficult to measure on small particles. They recommended light microscopy for use only as a preliminary tool for the analysis of consumer talc. Their detection limits for XRD analysis of consumer talcum products were as low as 0.1% by weight for tremolite, 0.25% for chrysotile but only 2.0% for anthophyllite. They concluded that the unique characterization of amphibole fibers (anthophyllite and tremolite versus fibrous talc) required TEM structural analysis (selected area electron diffraction — SAED) and micro-chemical characterization. Rohl and Langer recommended both XRD and TEM with SAED for analysis of consumer talc for their asbestos fiber content.

In another article published in 1974, Rohl (8) remarked, "Talc deposits include asbestos minerals such as chrysotile and amphiboles that may be carried over into consumer products. Optical [light] microscopy and X-ray diffraction analyses may not reveal their presence." Rohl reported that even at the detection limit for chrysotile by XRD (0.25%), there would be about a billion (10^9) fibers per mg of talc. He concluded that a sample of cosmetic talcum powder, which had been found negative for chrysotile when checked only by XRD, might contain billions of fibers that could be released during dusting with a half-gram dose.

In 1976, Rohl and Langer (9) reported on their testing of 20 consumer products labeled as "talc" or "talcum powder," including body powders, baby powders, facial talcums and one pharmaceutical talc. Of those 20 products, 10 were found to contain detectable amounts of tremolite and anthophyllite, principally asbestiform. The samples were analyzed by XRD, PLM, scanning electron microscopy (SEM) and TEM equipped with energy dispersive X-ray spectroscopy (EDS) and SAED capabilities. The authors noted that while some asbestos was resolvable by light microscopy, most samples were too fine-grained, with particle dimensions too small for light microscopy. By comparing the results of PLM and quantitative XRD with those from TEM analysis, they noted that large numbers of fibers could go undetected when using only the less sensitive techniques of PLM and XRD.

In 1990, Kremer and Millette (10) published a TEM procedure for the analysis of powdered talc for asbestos that had been in use in the McCrone laboratory in Atlanta since 1985. The method began by preparing an aqueous suspension of talc treated with the wetting agent, methylcellulose. Particles were transferred to a TEM grid via the "drop mount" method, where a drop of the talc-water suspension is placed on a carbon-coated formvar grid. Asbestos fibers were identified based on morphology as seen in the TEM, crystal structure as determined by SAED and elemental composition using an EDS system. Elongated particles with parallel sides and an aspect ratio of greater or equal to 3:1 were counted. Fibrous particles that needed to be distinguished from asbestos were listed as enrolled talc, ribbon talc, antigorite, talc fragments, silica and iron oxide fibers, and organic additives such as perfumes that may crystallize as fibers or needle-shaped crystals. The published method had a theoretical detection limit of 0.00005% (10^{-5}) weight percent based on a fiber 3 µm long by 0.2 µm wide by 0.06 µm thick as an asbestos fiber thought to be representative at the time of the smaller asbestos fibers found in some talc.

For lack of better statistical information at the time in 1990, the publication stated a rule of thumb that the detection of five or more asbestiform minerals of one variety in an analysis constituted a quantifiable level of detection. Subsequent method development in the area of TEM analysis for asbestos has shown that the detection of less than five fibers in a sample can provide a statistically valid result.

Although SEM is used to monitor asbestos in several European countries, it is not accepted in the U.S. for any analysis method of asbestos in talc. Davis, 1991 (11) tried to use the SEM to differentiate asbestos fibers from non-asbestos fibers. They reported: "This proved impracticable to do subjectively with any degree of reproducibility and had to be abandoned..." (11).

EXISTING METHODS FOR TALCUM POWDERS

The two historical methods for the analysis of talcum powders for asbestos are known as the CTFA-J4-1 (12) and USP-Talc (13). They are not considered up-to-date and are in need of revision.

The CTFA-J4-1 stands for the "Cosmetic, Toiletry and Fragrance Association method for Asbestiform Amphibole Minerals in Cosmetic Talc" first published in 1971. Part 1 is an XRD method. If an amphibole mineral is detected at a level greater than 0.5%, then the sample must be analyzed by Part 2 using (light) microscopy coupled with dispersion staining. To be counted, the fibers must have at least a 5:1 aspect ratio, be less than 3 µm in diameter and less than 30 µm in length. The document states that TEM with SAED offers greater sensitivity, but that it was not included because it was not thought to be suitable for normal quality-control application (based on time of analysis, expertise required and expense of equipment).

USP-Talc refers to the existing U.S. Pharmacopeia (USP) talc monograph published before 1983, which includes a test for "Absence of Asbestos." The asbestos test (which is currently pending revision) began with either an infrared spectroscopy (IR) test (USP-191) or an XRD test (USP-941). If the result of the IR or XRD test is negative, then no further analysis is required. If the IR or XRD test option gives a positive result, then an optical microscopy test (USP-776) must be done to confirm asbestos. The optical microscopy procedure does not require the use of polarized light.

SUMMARY OF A METHOD FOR THE ANALYSIS OF TALCUM POWDER FOR ASBESTOS

The method for the investigation for asbestos in talc described here is based on the early work of Walter and Lucy McCrone, the work of Kremer and Millette published in 1990 and the subsequent asbestos analytical procedures for PLM developed for the EPA, and the TEM methods standardized and published by the ASTM International (formerly American Society for Testing and Materials).

In the asbestos-talc method presented here, the sample is initially examined under a stereomicroscope at magnifications ranging from 7X to 40X. Portions of the particulate found in the sample are mounted in appropriate Cargille refractive index liquids for analysis by PLM using a polarized light microscope with a magnification range from 100X to 1,000X. The PLM analysis follows the procedures for bulk analysis of building materials described in the EPA 1993 bulk method (14). General SEM imaging of the sample using a scanning electron microscope can be done as an option to judge the extent of fibers in the sample. As a screening, XRD analysis is performed by scanning over a range of 3° to 45° 2Θ using 40kV, 25mA Cu Kα radiation. Mineral phases are identified with the aid of computer-assisted programs accessing a CD-ROM powder diffraction database. Mineral concentrations are based on relative peak heights and reference intensity ratios.

A transmission electron microscope equipped with EDS X-ray analysis system and capable of SAED is used to analyze the talc and asbestos fibers in the sample including tilting of talc/anthophyllite fibers. The TEM asbestos fiber counting criteria of fibers greater than 0.5 micrometer in length with at least a 5:1 aspect ratio as described in the Asbestos Hazard Emergency Response Act (AHERA) (15) and ASTM methods: D6281 (16), D5755 (17), D5756 (18) and D6480 (19) as well as in ISO 10312 (20) and 13794 (21) are used. The d-spacing/interfacial angle tables of Shu-Chun Su (22) are used when the option to index zone-axis patterns of amphibole minerals obtained by SAED in the TEM is chosen. The results of the TEM analysis are recorded using the procedures described in ASTM D6281.

TEM NOTES

The procedures for counting asbestos fibers with TEM described in ASTM D6281 and ISO 10312 (which are essentially the same) are the most fully developed of any of the TEM methods. The major difference between ASTM D6281 and ISO 10312 is that D6281 contains inter-laboratory precision data. Both methods have been vetted, debated and approved through the ASTM International or International Standards Organization procedures involving multiple ballots by experienced and knowledgeable scientists. Although ASTM D6281 and ISO 10312 were published as methods for asbestos in air, the basic counting procedures are the same for any sample once that sample material has been placed on a TEM grid. Since they are the most developed methodologies and have been accepted internationally, D6281 was chosen as the basis for the TEM part of this talc analysis method.

TABLE 1 Examples of the Minimum Number of Grid Openings Required to Achieve a Particular Analytical Sensitivity for a Collection Filter Area of 385 mm² and TEM Grid Openings of 85 µms (0.0072 mm²)

Analytical Sensitivity	Volume of Air Sampled, L						
Structures/L	500	1000	1200	2000	3000	4000	5000
0.1	1066	533	444	267	178	134	107
0.2	533	267	223	134	89	67	54
0.3	356	178	148	89	60	45	36
0.4	267	134	112	67	45	34	27
0.5	214	107	89	54	36	27	22
0.7	153	77	64	39	26	20	16
1.0	107	54	45	27	18	14	11
2.0	54	27	23	14	9	7	6
3.0	36	18	15	9	6	5	4
4.0	27	14	14	7	5	4	4
5.0	22	11	13	6	4	4	4
7.0	16	8	7	4	4	4	4
10.0	11	6	5	4	4	4	4

Figure 1. Table 1, reprinted from ASTM D6281-09 Standard Test Method (16), contains examples of the minimum number of grid openings required for certain analysis situations, ranging from four to 1,066 openings.

In both ISO 10312 and D6281 methods, one sentence has been interpreted by one scientist as indicating that the method is presumptive of asbestos present. The claim is that the fibers determined during the analysis using the method cannot be considered to be asbestos unless bulk analysis has been performed previously and asbestos identified in a product. This is not the case. The sentence contains two independent phrases that describe the applicability of the method. The first phrase describing the application of the method is for "the measurement of airborne asbestos in a wide range of ambient air situations." This expression is general, and there is absolutely no suggestion contained within it that asbestos is presumed to be present or presumed to be absent. The second phrase in the sentence is "for detailed evaluation of any atmosphere in which asbestos structures are likely to be present." This second phrase was intended to show an example of one of the many types of situations where the method might be used. D6281 is applicable for a detailed evaluation of any atmosphere for asbestos.

Number of Grid Openings to Be Counted

It is clear from examination of the equation used to calculate the concentration of asbestos fibers in a sample that the level of analytical sensitivity improves with the number of grid openings analyzed. ASTM D6281 does not specify a maximum number of grid openings that should be examined. Table 1 (see Figure 1) of D6281 contains examples of the minimum number of grid openings required for certain analysis situations that range from four to 1,066 openings. While the "rule of thumb" guideline of using 10 full-grid openings represents a judicious compromise between a reasonable experimental effort and a fairly low value of the detection limit, using two or more TEM grids (to analyze more grid openings) reduces the detection limit further and improves the precision of the estimates (23).

Differentiation of Asbestos Fibers from Non-asbestos Fibers

In 1990, Wylie (24) published some suggested characteristics of a population of particles with the asbestiform mineral habit. These included a mean aspect ratio of 20:1 or greater for fibers longer than 5 µm. Asbestos was characterized by very thin fibrils, usually less than 0.5 µm in width, and two or more of the following:

- Parallel fibers occurring in bundles
- Fiber bundles displaying splayed ends
- Fibers in the form of thin needles
- Matted masses of individual fibers
- Fibers showing curvature

Subsequently, the draft EPA R-93 (14) repeated most of the characteristics in a glossary providing a definition of a population of asbestos fibers as observed with light microscopy in a bulk sample. The EPA draft deleted the characteristic of fibers in the form of thin needles as being indicative of asbestiform.

TABLE 2-2. OPTICAL PROPERTIES OF ASBESTOS FIBERS

Mineral	Morphology and Color[1]	Refractive Indices[2] α	Refractive Indices[2] γ[5]	Birefringence[6]	Extinction	Sign of Elongation
Chrysotile (asbestiform serpentine)	Wavy fibers. Fiber bundles have splayed ends and "kinks". Aspect ratio typically >10:1. Colorless[3]	1.493-1.546 1.532-1.549 1.529-1.559 1.544-1.553	1.517-1.557 1.545-1.556 1.537-1.567 1.552-1.561	0.004-0.017	Parallel	+ (length slow)
Amosite (asbestiform grunerite)	Straight to curved, rigid fibers. Aspect ratio typically >10:1. Colorless to brown, nonpleochroic or weakly so.[4] Opaque inclusions may be present	1.657-1.663 1.663-1.686 1.663-1.686 1.676-1.683	1.699-1.717 1.696-1.729 1.696-1.729 1.697-1.704	0.021-0.054	Usually parallel	+ (length slow)
Crocidolite (asbestiform riebeckite)	Straight to curved, rigid fibers. Aspect ratio typically > 10:1. Thick fibers and bundles common, blue to dark-blue in color. Pleochroic.	1.693 1.654-1.701 1.680-1.698	1.697 1.668-1.717 1.685-1.706	0.003-0.022	Usually parallel	- (length fast)
Anthophyllite-asbestos	Straight to curved fibers and bundles. Aspect ratio typically > 10:1. Anthophyllite cleavage fragments may be present with aspect ratios <10:1. Colorless to light brown.	1.598-1.652 1.596-1.694 1.598-1.674 1.6148[7]	1.623-1.676 1.615-1.722 1.615-1.697 1.6362[7]	0.013-0.028	Parallel	+ (length slow)
Tremolite-Actinolite-asbestos	Straight to curved fibers and bundles. Aspect ratio typically > 10:1. Cleavage fragments may be present with aspect ratios <10:1. Colorless to pale green	Tremolite 1.600-1.628 1.604-1.612 1.599-1.612 1.6063[7] Actinolite 1.600-1.628 1.612-1.668 1.613-1.628 1.6126[7]	Tremolite 1.625-1.655 1.627-1.635 1.625-1.637 1.6343[7] Actinolite 1.625-1.655 1.635-1.688 1.638-1.655 1.6393[7]	Tremolite 0.017-0.028 Actinolite 0.017-0.028	Parallel and oblique (up to 21°); Composite fibers show parallel extinction.	+ (length slow)

[1]Colors cited are seen by observation with plane polarized light.

[2]From references 2, 11, 12, and 18, respectively. Refractive indices for n_a at 589.3nm.

[3]Fibers subjected to heating may be brownish. (references 13, 14, and 15)

[4]Fibers subjected to heating may be dark brown and pleochroic. (references 13, 14, and 15)

[5]∥ to fiber length, except ⊥ to fiber length for crocidolite only.

[6]Maximum and minimum values from references 2, 11, 12, and 18 given.

[7]± 0.0007

Figure 2. Table 2.2, reprinted from EPA Test Method R-93 (14), suggests using an aspect ratio of 10:1 in distinguishing between asbestos and non-asbestos fibers when considering optical properties.

Although these mineralogical population characteristics serve as a useful index in screening products and materials that contain fibers that might cause asbestos disease, the criteria are not very useful when dealing with individual fibers. The characteristics of parallel fibers occurring in bundles, fiber bundles displaying splayed ends, matted masses of individual fibers and fibers showing curvature are not related to the disease causing potential of asbestos fibers. Microscope analysis of individual fibers found on air sample filters produced from standard reference amosite (grunerite) asbestos fibers found very few parallel fibers occurring in bundles, fiber bundles displaying splayed ends, matted masses of individual fibers or fibers showing curvature. Trying to use two or more of those mineralogical characteristics would result in misclassifying up to 80% of the asbestos fibers.

The aspect ratio (AR) of a fiber, as determined by dividing its length by its width, has been used in discriminating between asbestos and non-asbestos fibers. Table 2.2 (see Figure 2) in the draft EPA R-93 method suggests using an aspect ratio of 10:1 in distinguishing between asbestos and non-asbestos fibers when considering optical properties. However, while research has shown that a population of cleavage fragment particles has a smaller average AR than a population of commercial asbestos fibers, the AR distributions of the two populations overlap, and on an individual basis, some fibers can be classified either way. Research by Wylie (25) reported in 1985 showed that 50% of the fibers in a known amosite (grunerite) asbestos sample would not be counted if a 20:1 aspect ratio were used as a criterion. Comparison of the aspect ratio plots in the 1977 Bureau of Mines Circular (26) shows that a criterion of about 5:1 aspect ratio appears to be the best aspect ratio discriminator for asbestos versus non-asbestos fibers. The 5:1 aspect ratio is used in AHERA; ASTM methods D6281, D5755, D5756 and D6480; and ISO 10312 and 13794.

The width of the fiber was found in inter-laboratory testing by Harper (27) to be the best discriminator for asbestos fibers, and that using a criterion of width that is less than or equal to one micrometer provides the least number of false negatives when dealing with asbestos and non-asbestos fibers. At the time of this writing, this information has not been incorporated into any standard method.

Elemental Analysis

The X-ray elemental spectrum collected from individual fibers is compared to data collected from known asbestos minerals. It is noted that the elemental compositions of talc and anthophyllite can be very similar. Although NIST-standard anthophyllite contains a small amount of iron, end-member anthophyllite, which contains very low or non-detectable amounts of iron, is reported in a standard mineralogical text (28) and documented in at least one talc deposit (29).

Zone Axis Indexing

Using ASTM D6281 allows for the option of indexing a portion of the SAED patterns and then comparing the values determined to calculated zone axis values. This is not possible with all fibers. Method D6281 (or any other TEM asbestos method) does not dictate the tolerance required for a positive match between observed and calculated values. Because of the known variability among the same mineral types found in different sources, it has been suggested that a tolerance of 10% might be used. Testing in the 1970s at the EPA research laboratory of chrysotile asbestos fibers from many sources showed that 5% tolerance was necessary when matching chrysotile asbestos SAED "d" values for the (002), (110) and inter-row spacing to account for the variability between different chrysotile fiber sources. This 5% criterion has been the standard taught during TEM asbestos analysis classes since 1987. This value is in line with early XRD data such as the 3.43% difference between the observed talc (002) measurement of 9.278 angstroms when compared to the calculated value of 8.96 angstroms by Gruner (30) and the 4.24% difference in the measured value for talc (002) by Gruner (30) of 8.960 angstroms and that measured by Stemple (31) of 9.34 angstroms. Table 4 (see Figure 3) in the draft Yamate document (23) shows a 16% difference between the d_1 of the SAED Internal Standard File Data and the d_1 from the X-ray Powder Diffraction File Data for the [101] zone axis for crocidolite (XRD File Index: 19-1061).

Talc Pseudo-Hexagonal Pattern

Table 4 in the draft Yamate document (23) lists [–1 4 2] as a reference zone axis for anthophyllite. With d_1 and d_2 both at 4.56 angstroms and an angle of 60°, this pattern is very close to the zone axis measured on a typical pseudo-hexagonal pattern obtained from a talc plate. Therefore, a fiber cannot be considered to be anthophyllite on the basis of a zone axis index match of the [–1 4 2] alone. Fortunately, a talc fiber can be differentiated from an anthophyllite fiber because the talc pattern remains evident as the talc particle is tilted, but the pattern changes when an anthophyllite fiber is tilted.

TABLE 4. COMPARISION OF d-SPACINGS FROM SAED FILE AND POWDER DIFFRACTION FILE (EXAMPLE)

		Internal Standard File Data				Powder Diffraction File Data (1975)		
Amphibole type	Zone axis	d_1 (Å)	d_2 (Å)	θ (deg)	Interrow spacing, R (Å)	d_1 (Å)	d_2 (Å)	File index no.
Amosite	[100]	5.3	9.14	90.0	5.3	5.22	9.20	17-725
	[30$\bar{1}$]	1.79	9.26	84.0	—	1.76	9.20	17-725
	[101]	4.88	9.23	74.0	5.17	4.84	9.20	17-725
	[$\bar{1}$01]	4.14	9.11	78.0	4.21	4.10	9.20	17-725
	[$\bar{3}$10]	5.22	5.13	95.0	—	5.22	5.12	17-725
Crocidolite	[100]	5.22	8.97	90.0	5.22	5.20	9.02	19-1061
	[101]	4.94	9.05	75.0	5.19	5.89	9.02	19-1061
	[$\bar{1}$10]	4.79	8.19	79.0	5.23	4.89	8.40	19-1061
	[30$\bar{1}$]	1.75	8.97	83.5	—	1.76	9.02	19-1061
	[$\bar{3}$10]	5.12	5.12	96.0	—	—	—	19-1061
Tremolite	[100]	5.04	9.03	90.0	—	5.07	8.98	13-437
	[101]	4.83	9.03	75.0	—	4.87	8.98	13-437
	[$\bar{2}$0$\bar{1}$]	2.59	8.97	80.5	—	2.59	8.98	13-437
	[30$\bar{1}$]	1.72	8.98	83.5	—	1.69	8.98	13-437
Anthophyllite	[100]	—	—	90.0	5.24	5.28	8.90	9-455
	[$\bar{1}$42]	4.56	4.56	60.0	—	4.50	4.50	9-455

Figure 3. Table 4, reprinted from the EPA Draft Report Contract #68-02-3266 by Yamate et al. (23), shows a 16% difference between the d_1 of the SAED Internal Standard File Data and the d_1 from the X-ray Powder Diffraction File Data for the [101] zone axis for crocidolite (XRD File Index: 19-1061).

Fibers with Kinks

When using the zone-axis indexing option, a few rare fibers with kinks in them that would normally be dismissed as talc ribbons by morphology may show a zone axis that match anthophyllite. Because the crystal structure matches anthophyllite and the fiber has substantially parallel sides for the majority of the fiber length, the fiber is counted as anthophyllite in this method.

RESULTS FROM USING THIS TALC METHOD

The method described here has been used to analyze both vintage talcum powders and some currently available. The analyses of samples of one brand of vintage talcum powder by this method showed the presence of asbestos fibers was described in Gordon (32). Analyses of one modern talcum powder product and a set of current cosmetic talc source samples from one

supplier using the same method did not detect any asbestos present. These later findings with the modern talcum powder are consistent with the results of a recent FDA sponsored study. During 2011–2012, the FDA contracted with AMA Analytical Services, Inc. to examine 28 cosmetic-grade talc samples from four suppliers and examine 34 off-the-shelf cosmetics for asbestos (33). Samples were received from suppliers who voluntarily sent samples; off-the-shelf samples were purchased directly from various stores based on a list of products determined by the FDA. AMA used a modified version of the New York State ELAP method 198.6/198.4 (non-friable bulk samples by PLM and TEM [34, 35]). AMA did not detect asbestos in any of the 28 talcs provided in 2011 from the suppliers or in 34 the talc-containing cosmetic products that were purchased in stores during the same period. In fact, AMA reported that all the talc materials tested contained only talc plates and no fibrous particles. Therefore, no specific testing procedures such as dispersion staining for PLM or SAED/EDS for TEM were needed. The limit of detection for the PLM portion of the AMA testing was based on one point out of 400 points multiplied by any loss during gravimetric reduction. Because there wasn't much loss for talcum powder samples, the PLM detection was reported as "around 0.21% to 0.23%." The AMA reported a limit of detection for TEM of "about 0.0000020% to 0.0000030%" based on the equation: (EFA x DF x M)/(AA x IM), where M was the mass of the smallest countable chrysotile asbestos fiber (1.60 x 10–15 grams), EFA was the effective filter area, DF was the dilution factor, AA was the area analyzed and IM was the initial sample mass. The result of the equation was multiplied by 100, to convert it to a percentage.

DISCUSSION

The methodology presented here updates the 1990 publication by Kremer and Millette and provides some information that may be helpful in updating the USP talc method. The analysis of talc powder for asbestos is most appropriately done with a combination of PLM, TEM and in some cases a screening by XRD. Low levels of asbestos fibers in talc, especially those too thin to be seen by light microscopy, may only be seen using the TEM analysis.

In 2014, Block et al. (36) discussed the modernization of the asbestos testing required in the USP talc monograph. The U.S. Food and Drug Administration (FDA) through the FDA Monograph Modernization Task Group asked the USP and National Formulary (USP-NF) to modernize the USP talc monograph in November 2010. This FDA request included updating the monograph to assure that talc used for cosmetic and pharmaceutical products is not sourced from mines that are known to contain asbestos, and asked that USP consider revising the current tests for asbestos to ensure adequate specificity. The expert panel that was charged with modernizing the USP talc monograph by the USP-NF recommended that the revision of the test for "Absence of Asbestos" omit the IR test and include a revised XRD procedure, in combination with one or more microscopic evaluations (PLM, TEM or SEM). The expert panel determined that the IR and XRD methods, as currently written, could lead to false-negative results, which could allow talc samples with asbestos contamination to pass. The panel also found that even with the additional light optical microscopy test (which currently does not include PLM), the analyst could not rule out the presence of hazardous fibers in the talc sample. In addition, the lack of identification procedures in the light optical microscopy section could lead to false-positive results. The 2014 report concluded that there was a need to modernize the current USP monograph because both the IR and the XRD methods have relatively high detection limits for asbestos, and there is no known "safe" level of asbestos exposure.

DISCLOSURE

The author has worked for both plaintiffs and defendants in lawsuits involving asbestos contamination. No client funds were received for the writing of this research article.

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MICROSCOPY COURSES

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a not-for-profit corporation

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FEATURED MCCRONE MICROSCOPY COURSES

Advanced Asbestos Identification
July 20–24, 2015
In this advanced course, using polarized light microscopy (PLM), students learn to identify and differentiate among all asbestos fibers and fibrous substitutes through review of basic theory and inclusion of more advanced methods.

Forensic Dust Analysis
July 27–31, 2015
This introduction to dust trace evidence begins with the history of dust analysis and the work of Locard, Popp, Schneider, Heinrich, Frei-Sulzer and others. The course will also explore techniques for collecting, separating, analyzing and interpreting dust evidence.

Sample Preparation and Manipulation for Microanalysis
July 27–31, 2015
The course will consist of lectures, demonstrations and hands-on training in a variety of techniques of small-particle handling. Students are welcome to bring problems and samples for discussion, practice and analysis.

Advanced Indoor Air Quality: Advanced Fungal Spore Identification
August 19–21, 2015
This course is designed for working analysts with moderate experience and goes beyond the basics to deal with the problems encountered on the job. It identifies less common ascospores, basidiospores, mitospores and other spores using current and classic mycological literature. Some important genera such as Cladosporium and Aspergillus will be examined at the species level.

Microscope Cleaning, Maintenance and Adjustment
August 26–27, 2015
Students will learn how the microscope works, various approaches to lens cleaning, Köhler illumination adjustment and other tricks of the trade. Lectures will be followed by hands-on adjustment and cleaning.

Animal Hair Identification
September 1–3, 2015
This course begins with an introduction to mammalian taxonomy, and the importance of establishing reference collections and hair atlases. The structural, morphological and anatomical features of hairs will also be covered.

Digital Imaging and Photomicrography
September 14–16, 2015
Students will learn how to capture better photomicrographs and use digital processing techniques to improve them. The course covers important aspects of digital imaging microscopy, including camera and microscope hardware, system set-up, user settings, collection of quality images, storage and printing.

Raman Microscopy
September 28–30, 2015
Emphasis is placed on applications of Raman that arise from its ability to characterize molecular structure, its general ease of use and minimal sample preparation requirements. Non-routine applications will also be discussed.

Visit *www.mcri.org* for a complete course calendar, full course descriptions and online registration.

Exhibit 19

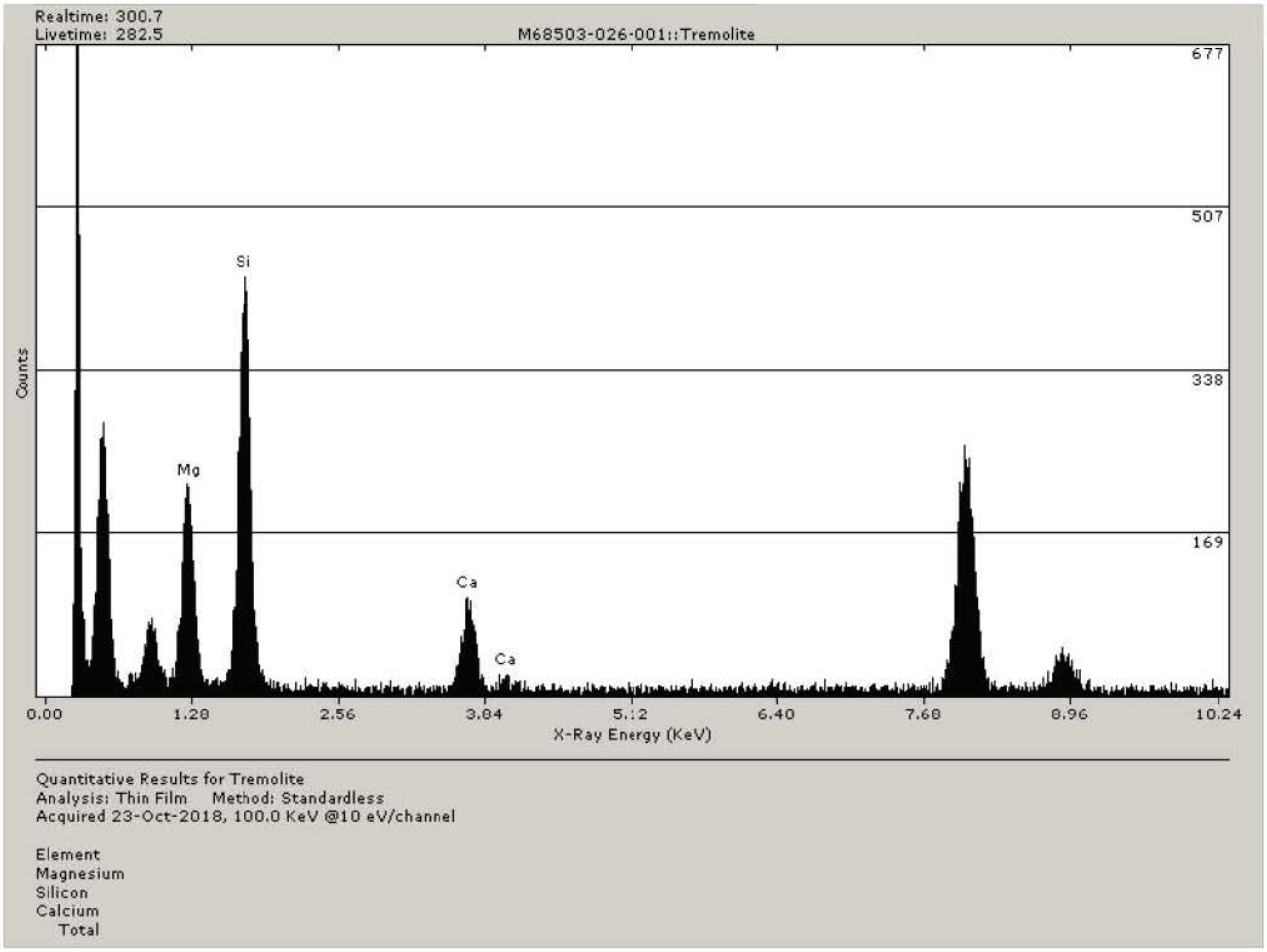
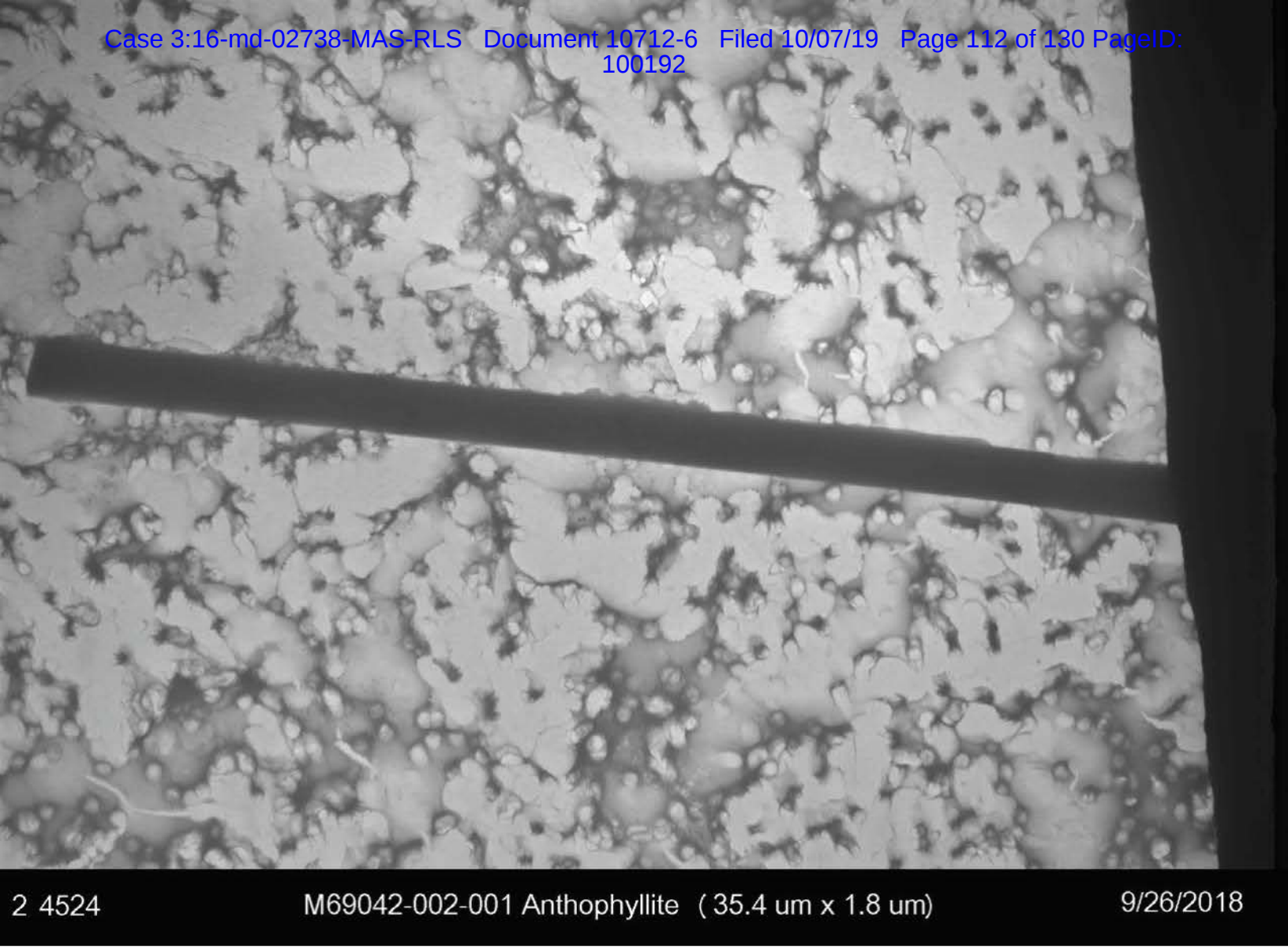
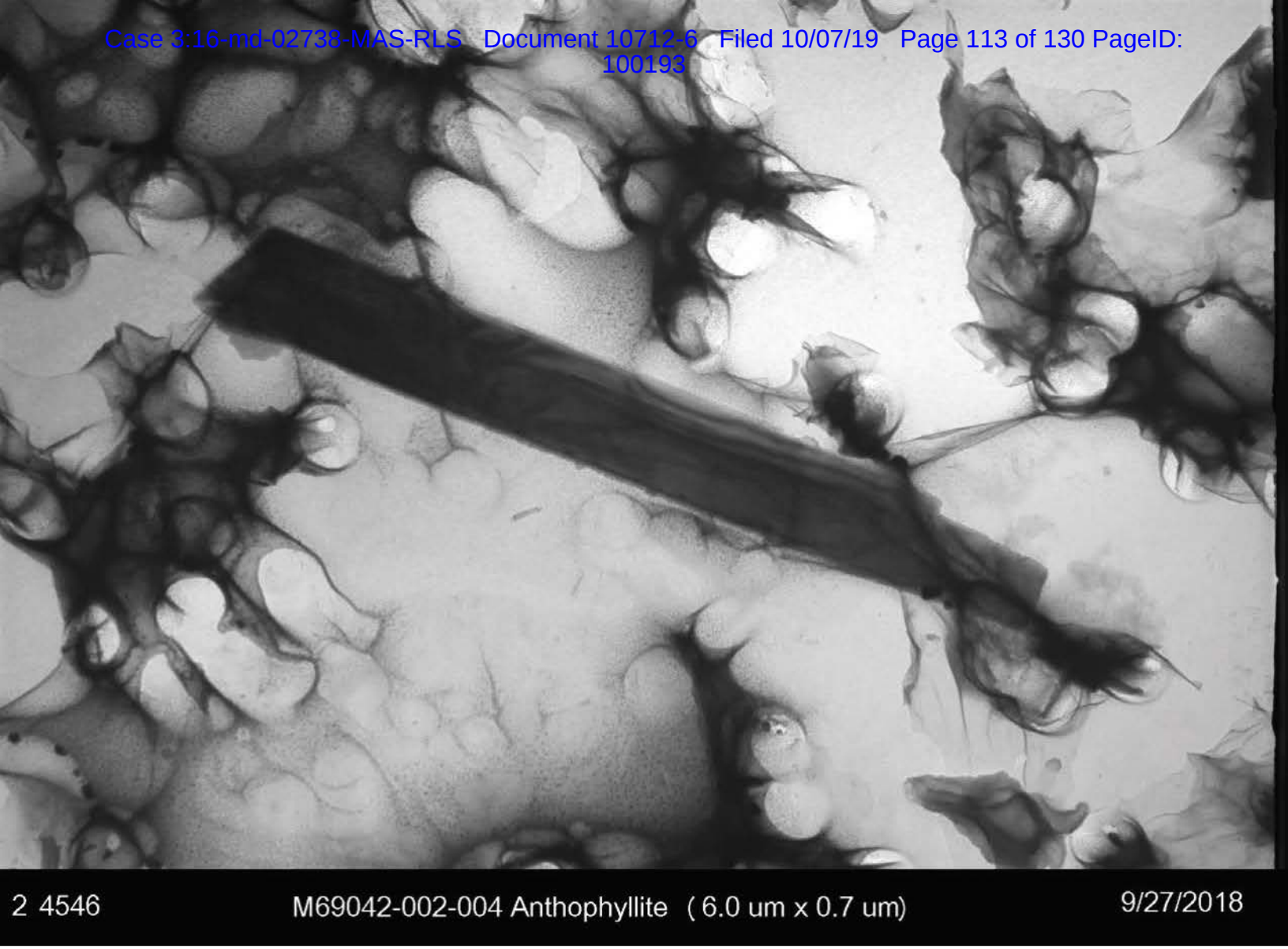
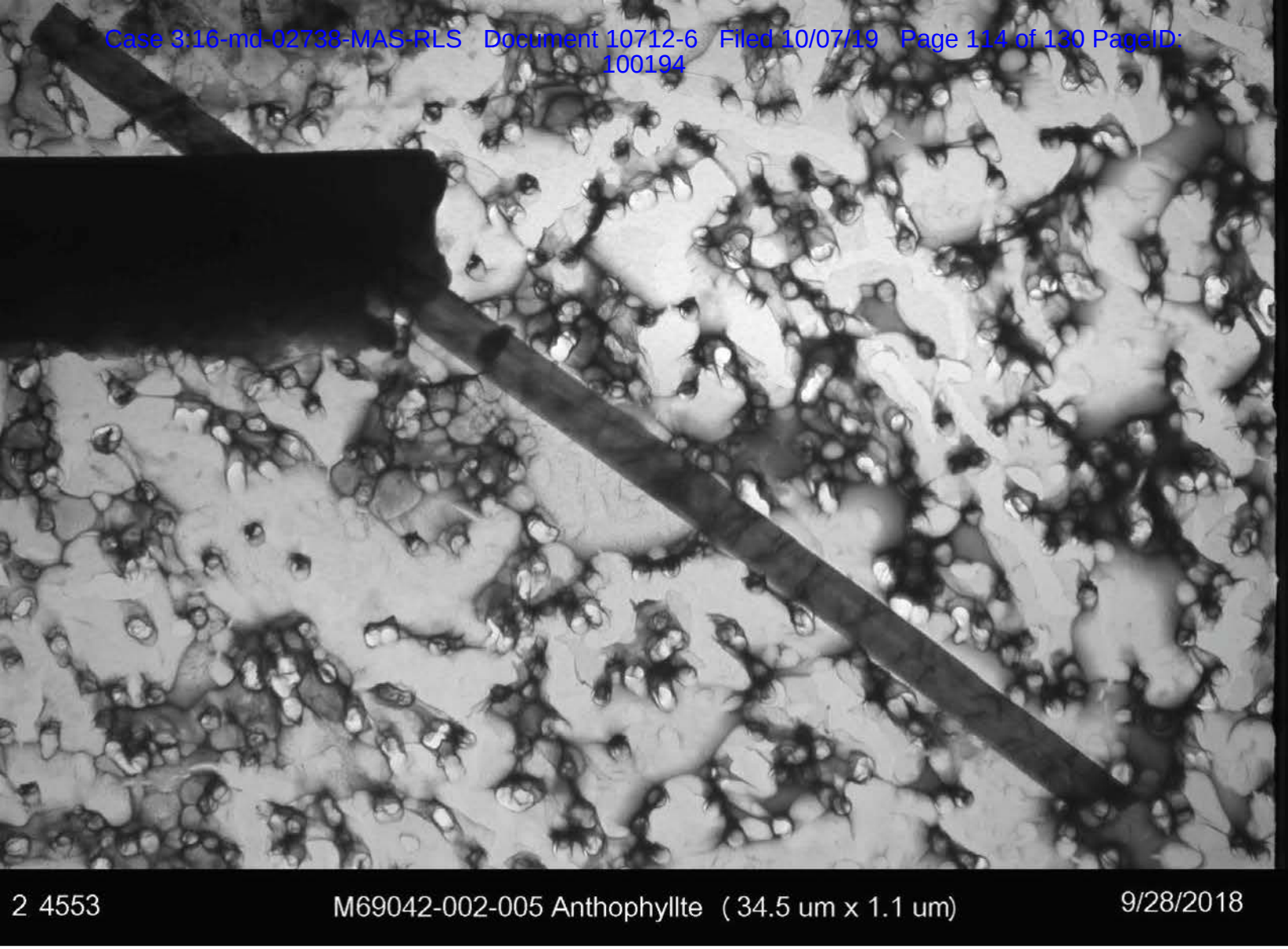

Realtime: 300.7
Livetime: 282.5
M68503-026-001::Tremolite
677
507
338
169
Counts
Si
Mg
Ca
Ca
0.00
1.28
2.56
3.84
5.12
6.40
7.68
8.96
10.24
X-Ray Energy (KeV)
Quantitative Results for Tremolite
Analysis: Thin Film Method: Standardless
Acquired 23-Oct-2018, 100.0 KeV @10 eV/channel
Element
Magnesium
Silicon
Calcium
Total

Exhibit 20

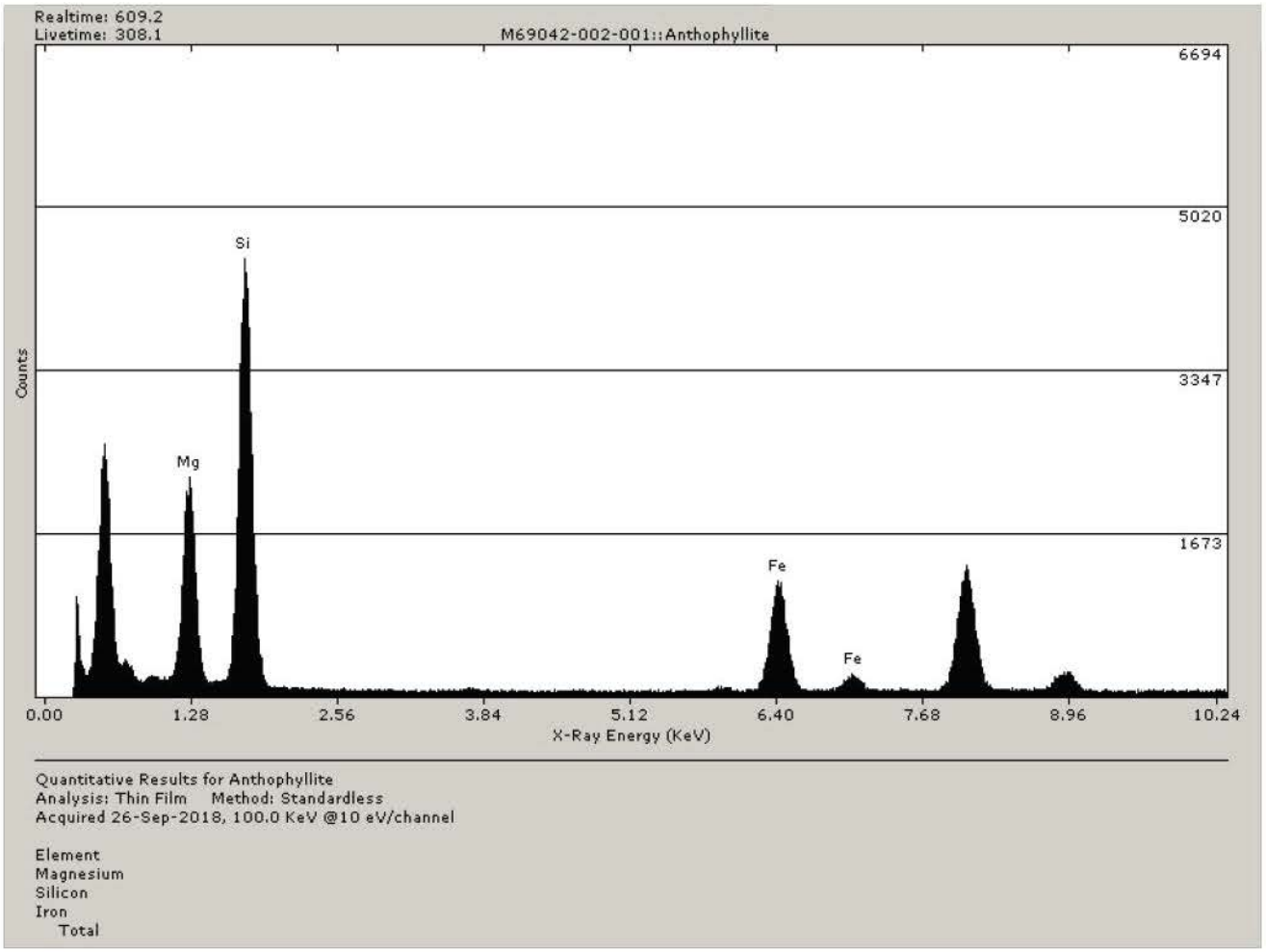

2 4524
M69042-002-001 Anthophyllite (35.4 um x 1.8 um)
9/26/2018


2 4546
M69042-002-004 Anthophyllite (6.0 um x 0.7 um)
9/27/2018

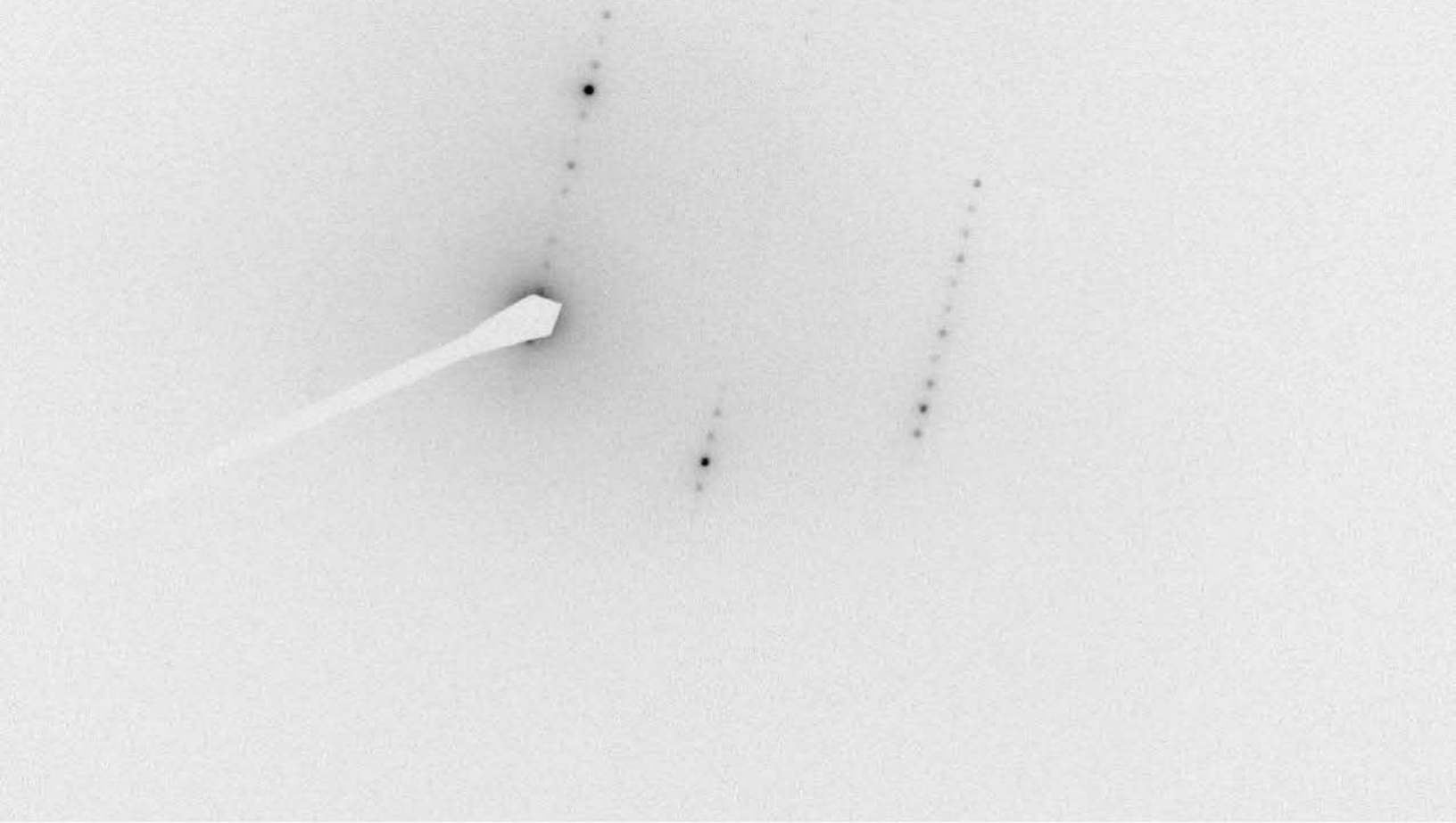

2 4553
M69042-002-005 Anthophyllte (34.5 um x 1.1 um)
9/28/2018

TEM Bulk Talc Structure Count Sheet						
Project/ Sample No.	M69042-002		Grid Box #	8621	No. of Grids Counted	2
Analyst:	Anthony Keeton			Length	Width	G. O. Area
Date of Analysis	9/26/2018 - 9/28/2018 &10/27/2018		G. O. in microns =	105	105	11025
Initial Weight(g)	0.02000			105	105	11025
Analysis Type	Post Separation Talc Analysis		Grid Acceptance	Yes	Average	11025
Scope No.	Accelerating Voltage	100 KV	Loading%	12%	G.O.s Counted	100
2	Screen Magnification	20 KX	Area Examined mm²			1.103

Str. #	Grid Opening	Structure	Asbestos Type	Length	Width	Ratio	SAED	EDS
NSD	B2-B6							
NSD	B7							
1	B8	Bundle	Anthophyllite	35.4	1.8	19.7	X	X
2		Bundle	Anthophyllite	12.4	1.1	11.3	X	X
NSD	B9							
NSD	B10							
NSD	C3							
NSD	C4							
NSD	C5							
NSD	C6							
NSD	C7							
NSD	C8							
NSD	C9							
NSD	C10							
3	E1	Bundle	Anthophyllite	6.4	1.1	5.8	X	X
NSD	E2							
NSD	E3							
NSD	E4							
NSD	E5							
NSD	E6							
NSD	E7							
NSD	E8							
4	E9	Bundle	Anthophyllite	6	0.7	8.6	X	X
NSD	E10							
NSD	F1							
NSD	F2							
NSD	F3							
NSD	F4							
NSD	F5							
NSD	F6							
NSD	F7							
NSD	F8							
NSD	F9							
NSD	F10							
NSD	G1							
NSD	G2							
NSD	G3							
NSD	G4							
NSD	G5							
NSD	G6							
NSD	G7							
NSD	G8							
NSD	G9							
NSD	G10							
NSD	H3							
NSD	H4							
NSD	H5							
NSD	H6							
5	H7	Bundle	Anthophyllite	34.5	1.1	31.4	X	X
NSD	H8							

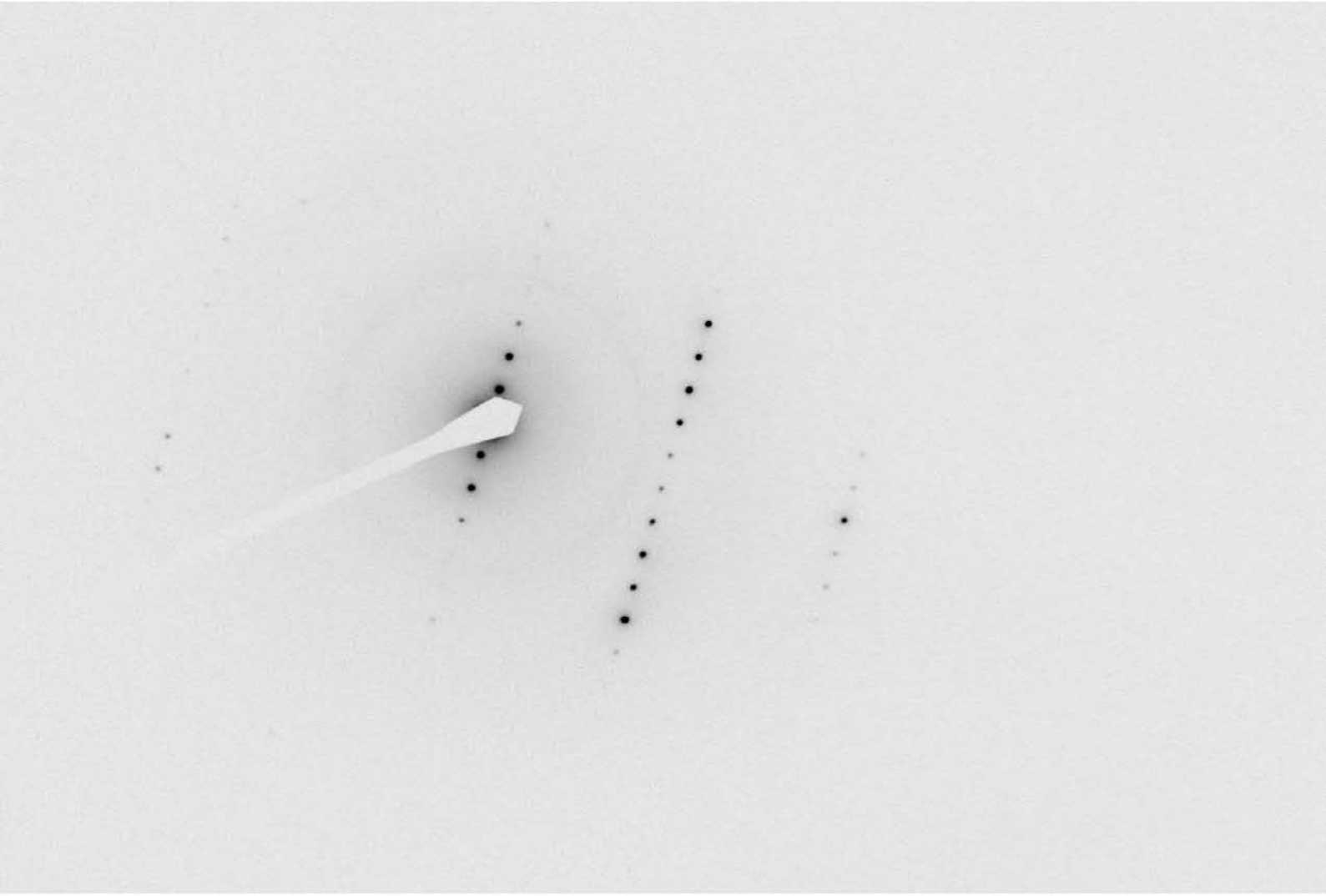

Realtime: 609.2
Livetime: 308.1
M69042-002-001::Anthophyllite
6694
5020
3347
1673
Counts
Si
Mg
Fe
Fe
0.00
1.28
2.56
3.84
5.12
6.40
7.68
8.96
10.24
X-Ray Energy (KeV)
Quantitative Results for Anthophyllite
Analysis: Thin Film Method: Standardless
Acquired 26-Sep-2018, 100.0 KeV @10 eV/channel
Element
Magnesium
Silicon
Iron
Total

2 4520 M69042-002-001 Anthophyllite Diffraction - 1 @ 50cm 9/26/2018



2 4521 M69042-002-001 Anthophyllite Diffraction - 2 @ 50cm 9/26/2018

Exhibit 21



2 4680 M68503-026-001 Tremolite Diffraction @ 50cm 10/23/2018

Exhibit 22

Johnson & Johnson

J&J Consumer Companies Worldwide Specification

Issued

Strictly Confidential

ANALYSIS OF POWDERED TALC FOR ASBESTIFORM MINERALS BY TRANSMISSION ELECTRON MICROSCOPY

Name	Type
TM7024	Test Method

Revision	1	Owner	Corporate
Issued Date	1995-08-21	Expiration Date	9999-12-31
Geographical Scope	Local	Specification Category	Permanent
Security Classification		Review Interval (Months)	0

Related Information

Template	Test Method Global	SCO	
Co-Owners		Owning Region	North America

Revisions

Name	Rev	State	Description of Change	Reason for Change	Owner	Issued Date	Expiration Date
TM7024	1	Issued			Corporate	1995-08-21	9999-12-31

Approvals

Signer	Role	Organizations	Date/Time
No Objects Found			

Content

Name	Format	File Size
TM7024.doc	generic	35840

Reference Documents

Name	Description

No Objects Found

Related Specifications

Name	Type
No Objects Found	

User Defined Attributes

No Objects Found

Additional Attributes

No Objects Found

Test Method

Company:

- [ ] *Personal Products Worldwide*
- [ ] *Personal Products Company*
- [ ] *Desbiens Products Inc.*
- [ ] *Johnson & Johnson Products Inc.*
- [ ] *Odonto Corporation Ltd.*
- [X] ***Johnson & Johnson Consumer Products Co.***

Document No.: TM7024 **Franchise:** **Location:** ROYSTON, FLUID, KOLMAR

Document Type: Permanent **Expiration Date:** None

Subject: ANALYSIS OF POWDERED TALC FOR ASBESTIFORM MINERALS BY TRANSMISSION ELECTRON MICROSCOPY

REVISION	AUTHORIZATION	DESCRIPTION OF CHANGE
03/08/89	BCR011362	New Test method.
03/21/95	CR020127	Location revised. (Spec. Dept.)
08/21/95	CR020688	Location revised. (Spec. Dept.)

Test Method

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Document No.: TM7024 **Franchise:** **Location:** ROYSTON, FLUID, KOLMAR

Document Type: Permanent **Expiration Date:** None

Subject: ANALYSIS OF POWDERED TALC FOR ASBESTIFORM MINERALS BY TRANSMISSION ELECTRON MICROSCOPY

1.0 SCOPE & PURPOSE

This method is applicable to the identification and quantitation of small (typically 1-20 micrometer) asbestiform minerals in powdered talc. Samples may be previously screened with light microscopy or x-ray diffraction techniques.

2.0 PRINCIPLE OF METHOD

The combined techniques of transmission electron microscopy (TEM), selected area electron diffraction (SAED) and energy dispersive x-ray analysis (EDXRA) permit the detection of asbestiform minerals based on morphological characteristics, followed by a definitive mineralogical identification of each fiber.

3.0 INTERFERENCES

Interferences are caused by fibrous particles which must be distinguished from positively identifiable asbestos, and by large particles or particle aggregates which may obscure fibers. Positively identified non-asbestos fibers include rolled talc, ribbon talc, antigorite, silica fibers and iron oxide fibers. Organic additives such as perfumes may crystallize out as fibers or needle-shaped crystals in finished cosmetic products. In the absence of positive identification, all other fibers must be classified as unidentifiable.

4.0 INSTRUMENTAL CONDITIONS

The talc specimen grids are examined in the TEM at an accelerating voltage of 120 kv and at magnification of 20,000X and 5,000X.

5.0 SENSITIVITY

This method is capable of detecting a single fiber as small as 1 micrometer (mm) long by 0.075 mm wide in the entire TEM field, which results in a theoretical detection limit of 10^{-5} weight percent. Such fibers usually can be identified readily by SAED and EDXRA. The mass of a fiber with the above dimensions is 1.1×10^{-14} g for chrysotile and 1.5×10^{-14} g for amphibole.

6.0 LIMIT OF QUANTIFIABLE DETECTION

The detection of five or more asbestiform minerals of one variety in an analysis constitutes a quantifiable level of detection. When no asbestiform minerals are detected, a representative fiber size is used to calculate a detection limit. A representative fiber size is 3 mm long by 0.2 mm wide by 0.06 mm thick, which is considerably larger than the smallest fiber that can be detected (see section 5, SENSITIVITY), but is more typical of small asbestos fibers that are detected in talc analyses. The mass of five such fibers is calculated as follows:

3 mm x 0.2 mm x 0.06 mm = 0.036mm^3 per fiber
x 3.3E-12 g / mm^3 = 1.2 E-13 g per fiber

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Document No.: TM7024 **Franchise:** **Location:** ROYSTON, FLUID, KOLMAR

Document Type: Permanent **Expiration Date:** None

Subject: ANALYSIS OF POWDERED TALC FOR ASBESTIFORM MINERALS BY TRANSMISSION ELECTRON MICROSCOPY

x 5 fibers = 6E-13 grams per 5 fibers.

The limit of quantifiable detection for most talc analyses is approximately 6 x 10^{-4} weight percent. The theoretical and quantifiable detection limits assume homogeneity of the material being sampled.

7.0 QUALITY ASSURANCE

Blank suspensions are routinely prepared and tested in order to monitor potential residual contamination from the sample jars. Blank carbon-coated grids are routinely tested to monitor the ambient fiber count. If greater than 4 fibers per grid are present, the jars are pre-cleaned or new carbon-coated grids are prepared, respective of the test.

8.0 BACKGROUND CORRECTION

As of the time of this writing, background correction has not been necessary. The amount of background asbestos detected has been insignificant in comparison to the levels of asbestos found in contaminated samples.

9.0 PREPARATION AND ANALYSIS TIME

Preparation time per sample (including preparation of related materials) is one hour. Analysis search time per sample is a maximum of two hours.

10.0 APPARATUS

10.1 Analytical balance with 0.0001 gram sensitivity

10.2 Weighing boats

10.3 Narrow spatula

10.4 Wide mouth polyethylene jars (125 ml)

10.5 Mild ultrasonic bath, minimum 50 watts

10.6 Micropipettor (5-10 ml range) with disposable tips

10.7 Standard 3 mm diameter, 200 mesh, copper TEM grids, covered with a carbon-coated formvar film.

10.8 Transmission electron microscope (TEM) with an 80-120 kv accelerating voltage and energy dispersive x-ray analyzer.

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- [X] ***Johnson & Johnson Consumer Products Co.***

Document No.: TM7024 **Franchise:** **Location:** ROYSTON, FLUID, KOLMAR

Document Type: Permanent **Expiration Date:** None

Subject: ANALYSIS OF POWDERED TALC FOR ASBESTIFORM MINERALS BY TRANSMISSION ELECTRON MICROSCOPY

11.0 REAGENTS

11.1 Methyl cellulose, powder, USP 4000 cps - Fisher Certified Reagent #M-352 or equivalent

11.2 Water: deionized, particle free (+0.2 mm filtered)

11.3 Methyl cellulose solution: 0.002% (wt/vl) (20 ppm). Dissolve 20 % 0.5 mg of methyl cellulose in 500 ml of deionized particle free water to make a 0.004% stock solution. Dilute 1:1 to make a working solution.

NOTE: Methyl cellulose acts as a wetting agent to aid in maintaining a uniform particle distribution as the sample dries, by greatly reducing the surface tension of water.

12.0 SAMPLE PREPARATION

12.1 Transfer 30 to 50 mg of talc powder to a clean 125 ml polyethylene jar.

12.2 Add 80 ml of 20 ppm methyl cellulose solution, cap and shake vigorously for one minute.

12.3 After shaking, loosen cap and ultrasonicate for 10 minutes in order to disperse the finer particles. Then shake again for one minute to produce a uniform suspension.

12.4 Immediately after shaking, uncap and remove 9.2 microliters with a micropipette.

12.5 Transfer a 9 ml drop to a carbon film covered TEM grid. (Grid was first lightly anchored by 2 parallel strips of double-stick tape mounted 3 mm apart on a clean glass microscope slide.) Repeat to make two sample grids per talc sample.

NOTE: Do not expel the remaining 0.2 ml suspension from the micropipette tip. It tends to sputter and frequently destroys the stability of the sample drop.

12.6 Transfer slide with grids to a desiccator. (Drying time is 2-3 hours.) Do not leave the grids on the slide for more than one day as the double-stick tape may adhere too tightly.

NOTE: The talc:water ratio may need to be varied for some samples. Preparation of talc samples with a significantly finer or coarser particle size results in large differences in particle coverage on the TEM grid.

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Document No.: TM7024 **Franchise:** **Location:** ROYSTON, FLUID, KOLMAR

Document Type: Permanent **Expiration Date:** None

Subject: ANALYSIS OF POWDERED TALC FOR ASBESTIFORM MINERALS BY TRANSMISSION ELECTRON MICROSCOPY

13.0 TEM ANALYSIS

13.1 Definition of fiber: An elongated particle with parallel sides and an aspect ratio K3:1. The definition employed may vary with the needs of the client.

13.2 Scan sample at 120-150X magnification to check for even dispersion of particles and to locate grid squares with optimum particle density. (Optimum particle density is particle coverage over 15-35% of the field of view.)

13.3 Scan three grid squares on each grid at 20,000X magnification and seven grid squares on each grid at 5,000X for asbestiform minerals. Each asbestiform mineral is recorded as to type (chrysotile, tremolite, anthophyllite, etc.), structure (bundle, clump, fiber) and dimensions (length x width).

13.4 Questionable fibers are examined first by SAED. The chrysotile SAED pattern is unique and diagnostic. Amphibole SAED patterns are variable but usually characteristic. Additional analysis and measurement of amphibole SAED patterns are done if warranted.

13.5 Ten percent of chrysotile fibers are checked by EDXRA for further confirmation. If the SAED pattern is not clearly diagnostic, or if it is consistent with an amphibole SAED pattern, then it is examined by EDXRA to confirm the identification or to identify the type of amphibole.

14.0 CALCULATION OF RESULTS

14.1 Mass of chrysotile fibers: M(f)

$M(f) = \pi r^2 l \times d$

$\pi = 3.14159$

r = fiber radius

l = fiber length

d = density of chrysotile = 2.55×10^{-12} g/mm^3

14.2 Mass of asbestiform amphibole particles: M(a)

M(a) = l x w x th x d

l = length

w = width

th = thickness Z 0.3 width (approximation)

d = density of amphiboles = 3.3×10^{-13} g/mm^3

14.3 Mass of talc deposited on each TEM grid: M(s)

M(s) = T x (V/H)

T = amount of talc sampled (step 12.1)

V = volume of aliquot transferred to TEM grid (step 12.5)

Test Method

Company:

- [ ] *Personal Products Worldwide*
- [ ] *Personal Products Company*
- [ ] *Desbiens Products Inc.*
- [ ] *Johnson & Johnson Products Inc.*
- [ ] *Odonto Corporation Ltd.*
- [X] ***Johnson & Johnson Consumer Products Co.***

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H = volume of methyl cellulose solution (step 12.2)

14.4 Total estimated talc mass examined: M(t)

M(t) = M(s) x (N x A(s))/A(g)

N = number of grid squares examined

A(s) = area of a single TEM grid square

A(g) = area of an entire TEM grid (effective area over which a 9 microliter drop of suspension dries)

14.5 Weight percent:

$$\frac{\text{sum total of M(f) or M(a) x 100}}{\text{M(t)}}$$

15.0 CALCULATION OF A DETECTION LIMIT

15.1 M(dl) = A minimum quantifiable mass of asbestos fibers, based on the detection of 5 fibers (approximately 6E-13 grams, from Section 6).

15.2 Detection Limit (Weight Percent) = $\frac{\text{M(dl) x 100}}{\text{M(t)}}$

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